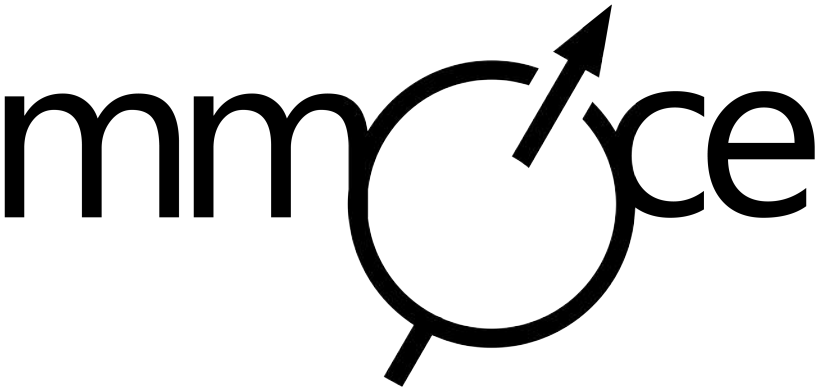
*M*agnetic *M*oments in *C*entral *E*urope 2015

February 25th-March 1st,
Krynica-Zdrój, Poland



Magnetic Moments in Central Europe 2015 Book of Abstracts

Krynica-Zdrój, Poland

2015-02-25 / 2015-03-01

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Publisher
Nobell Congressing sp. z o.o.
Nadbrzeżna 4
05-850 Ożarów Mazowiecki
Poland
www.nobell.pl

Printing
Mirage Hobby
ul. Tyniecka 36
02-621 Warszawa
Poland

Typeset with XeLaTeX and TeX Live 2013

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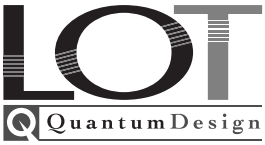
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Dear Colleagues and distinguished guests,

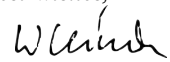
Welcome to the Magnetic Moments in Central Europe conference in Krynica, Poland.

The biennial conference Magnetic Moments in Central Europe (MMCE) was established in 2007 with the vision of providing a unique knowledge-sharing event for NMR scientists and students in the region. The current meeting is the fourth (fifth if we include MMCE number 0 in Ljubljana in 2008) in a series of successful events. The meetings in Otočec in Slovenia (2009), Tatranská Lomnica in Slovakia (2011), and Semmering in Austria (2013) proved the concept.

The main aim of this conference is to provide an effective forum for discussions, at both formal and informal levels. The number of registered participants should guarantee a stimulating environment, and the conference program allows maximizing the time the participants spend together.

During the past years, since its inception, the conference was sponsored by Varian, and then Agilent. This year, the unexpected and disastrous decision of Agilent to leave the NMR business affected not only the NMR community, but also made the organization of MMCE more demanding. Thanks to the generous support of Jeol, we were able to complete this task. We hope that this meeting will again provide us possibilities to share scientific experience and continue the good traditions of MMCE.

Best wishes,



Wiktor Koźmiński

Mission Statement

The conference Magnetic Moments in Central Europe (MMCE) was conceived in 2007 with the vision of providing a unique knowledge-sharing event for NMR scientists and students in the region. The philosophy behind MMCE is centered on the following main goals:

- The conference is didactic in its fundamental spirit. Rather than expecting scientists to communicate their latest results aimed to be published in a scientific journal, the conference wants to offer, in the form of so-called Tutorial Talks, scientists an opportunity to speak about their research, their intellectual and emotional struggles, their "a-ha!" moments, their provocative thoughts and their acquired wisdom in relation to their chosen topic in such an informal, conceptual, personal and edifying manner that would not normally be possible in the context of a "regular" scientific presentation. Tutorial Talks are expected to project a kind of distilled wisdom on a topic that can potentially be more captivating and instructive to both students and seasoned NMR scientists than a new-result-centered "regular" lecture.
- In addition to being beneficial for specialists in a given field, Tutorial Talks should foster communication and understanding between various branches of NMR.
- MMCE aims to address all walks of theoretical and applied NMR spectroscopy, ranging from fundamental theory through small-molecule structure determination, new pulse sequences, software and hardware development, etc. to biological molecules.
- The talks offered by a speaker at MMCE need not necessarily be "trendy"; old concepts addressed from a new, exciting, or eye-opening perspective are welcome.
- MMCE also accommodates the presentation of cutting-edge new results, with the speakers being encouraged to add some didactic flavor to their talks.
- MMCE wishes to act as a dynamic NMR discussion forum in both an intellectual and social sense. Discussions are encouraged after each presentation, and to that end sufficient room is provided in the program.
- It aims to become a well-consolidated platform for building social capital in the NMR community of Central Europe and beyond.
- MMCE is dedicated to involving many young scientists and university students as participants.

MMCE is held every second year in varying locations within Central Europe. It usually lasts for five days, starting Wednesday evening and ending Sunday noon. Topics are divided into sessions. There are no parallel sessions. As a general rule of thumb, each session starts with a Tutorial Talk lasting about 1 hr, followed by a few Invited Talks which are 30 min long. Tutorial Speakers and Invited Speakers are invited by the scientific board of MMCE. Each session also provides space for a few 20-minute talks delivered by the regular participants of the conference. There are also poster sessions and student presentation sessions.

General Information

Lectures

Oral presentations will take place in the Nubis Hall. Speakers should contact the technical assistant in the conference room before the session to ensure proper display of their presentations. The chairpersons are asked to ensure that presentations do not exceed the allotted time limit. Five-minute slots should be reserved for discussion and switching of presentations.

Posters

Please pin your posters to the boards in the Floral Hall (Sala Kwiatowa) on Thursday, February 26, using the provided pins. Posters are numbered by the page they appear in the abstract book (in alphabetical order). There are two poster sessions for *odd* (Thursday, 17:15–19:30) and *even-numbered* posters (Friday, 17:15–19:30). Presenting authors are asked to be present in front of their posters during the session. Posters should be removed after the dinner on Friday, February 27. After this time, the remaining posters will be discarded.

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Police	**997** or **112**
Ambulance	**999** or **112**

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`www.pogodynka.pl`	official service of the Institute of Meteorology and Water Management, numerical and synoptic forecast

Conference WWW Page

`nmr.cent3.uw.edu.pl/mmce2015`

Program Outline

Wednesday, February 25	
14:00	*Arrival Registration*
19:00	Wiktor Koźmiński
19:15	Csaba Szantay
20:00	*Dinner*

Thursday, February 26	
7:30	*Breakfast*
8:30	Isabella Felli
9:15	Bernhard Brutscher
9:45	Dennis Kurzbach
10:00	Thomas Schwarz
10:15	*Coffee break*
10:45	Klaus Zangger
11:30	Katalin Kövér
12:00	Lars Hanson
12:30	*Lunch*
14:00	Hiroaki Sasakawa
14:15	Michal Malon
14:45	Dušan Uhrín
15:15	Svetlana Simova
15:45	*Coffee break*
16:30	Christian Griesinger
17:15	**Poster session**
19:30	*Dinner*

Friday, February 27	
7:30	*Breakfast*
8:30	Harald Schwalbe
9:15	Zofia Gdaniec
9:45	Maja Marušič
10:00	Vojč Kocman
10:15	*Coffee break*
10:45	Peter Lukavsky
11:15	Yusuke Nishiyama
12:00	Piotr Paluch
12:15	Simon Glanzer
12:30	*Lunch*
14:00	Hartmut Oschkinat
14:45	Marek Potrzebowski
15:15	Gregor Mali
15:45	*Coffee break*
16:30	Christina Thiele
17:15	**Poster session**
19:30	*Dinner*

Saturday, February 28	
7:30	*Breakfast*
8:30	Jozef Kowalewski
9:15	Bert Heise
9:45	Zoltán Béni
10:15	*Coffee break*
10:45	Anna Zawadzka-Kazimierczuk
11:15	Ivana Biljan
11:45	Saurabh Saxena
12:00	Radek Pohl
12:15	*Lunch*
14:00	*Free time*
19:30	*Dinner*

Sunday, March 1	
7:30	*Breakfast*
9:00	Krzysztof Kazimierczuk
9:45	Vladimír Mlynárik
10:15	*Coffee break*
10:45	Władysław Węglarz
11:15	Mateusz Urbańczyk
11:30	Poster prize winner
11:45	Closing
12:15	*Lunch*
14:00	*Departure*

Detailed Program

Wednesday, February 25	
14:00	*Arrival* *Registration*
19:00	Wiktor Koźmiński University of Warsaw, Poland Opening
19:15	Csaba Szantay Gedeon Richter Plc., Hungary On the human aspects of scientific thinking in NMR spectroscopy
20:00	*Dinner*

Thursday, February 26

07:30	*Breakfast*
	Chairperson: Harald Schwalbe
8:30	Isabella C. Felli University of Florence, Italy New methods based on ^{13}C direct detection to study intrinsically disordered proteins
9:15	Bernhard Brutscher University of Grenoble, France Fast-pulsing NMR techniques: general concepts, practical aspects, and selected applications
9:45	Dennis Kurzbach University of Vienna, Austria Direct monitoring of correlated ensemble fluctuations in intrinsically disordered proteins
10:00	Thomas Schwarz University of Vienna, Austria MAP1B light chain and its interaction with microtubules
10:15	*Coffee break*
	Chairperson: Csaba Szantay
10:45	Klaus Zangger University of Graz, Austria Simplifying proton-detected NMR spectra by spatially-selective excitation
11:30	Katalin E. Kövér University of Debrecen, Hungary Precise measurement of heteronuclear coupling constants: novel applications of broad-band proton-proton decoupling
12:00	Lars Hanson Technical University of Denmark, Denmark Visualisation of basic NMR: quantum and classical aspects
12:30	*Lunch*
	Chairperson: Predag Novak
14:00	Hirosaki Sasakawa JEOL UK, United Kingdom Recent developments in hardware: the most sophisticated spectrometer and probes
14:15	Michal Malon JEOL Resonance Inc., Japan Application of AQARI (Accurate Quantitative NMR with Internal Substance) to quality control of organic reagents
14:45	Dušan Uhrín University of Edinburgh, United Kingdom New point of view in utilising tags for structural analysis of complex mixtures
15:15	Svetlana Simova Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Bulgaria From monosaccharides to polysaccharides: NMR applications
15:45	*Coffee break*
	Chairperson: Predag Novak
16:30	Christian Griesinger Max Planck Institute for Biophysical Chemistry, Germany NMR spectroscopy in structural biology and for drug validation and development in neurodegeneration
17:15	**Poster session**, odd-numbered posters
19:30	*Dinner*

Friday, February 27

07:30	*Breakfast*
	Chairperson: Janez Plavec
8:30	Harald Schwalbe Goethe University Frankfurt, Germany Investigating dynamic layers of cellular information transfer
9:15	Zofia Gdaniec Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poland Pitfalls in RNA structure prediction
9:45	Maja Marušič Slovenian NMR Center, National Institute of Chemistry, Slovenia Structural diversity of guanine-rich sequences in genomes of human papillomaviruses
10:00	Vojč Kocman Slovenian NMR Center, National Institute of Chemistry, Slovenia A tetrahelical DNA fold not stabilized by G-quartets
10:15	*Coffee break*
	Chairperson: Tibor Liptaj
10:45	Peter J. Lukavsky CEITEC, Masaryk University, Czech Republic Structural insights into aberrant splicing of CFTR exon 9
11:15	Yusuke Nishiyama JEOL Resonance Inc, Japan Application of homonuclear mixing on ^{1}H in 100 kHz magic angle spinning
12:00	Piotr Paluch Centre of Molecular and Macromolecular Studies, PAS, Poland 2D and 3D CP-VC as tools for dynamics study
12:15	Simon Glanzer University of Graz, Austria Real time J-scaling in nuclear magnetic resonance
12:30	*Lunch*
	Chairperson: TBA
14:00	Hartmut Oschkinat Leibniz-Institut für Molekulare Pharmakologie, Germany Protein MAS DNP at 190 K and MAS triple-resonance spectroscopy of membrane proteins
14:45	Marek Potrzebowski Centre of Molecular and Macromolecular Studies, PAS, Poland NMR crystallography
15:15	Gregor Mali National Institute of Chemistry, Slovenia Structural studies of metal-organic frameworks by solid-state NMR spectroscopy and first-principles calculations
15:45	*Coffee break*
	Chairperson: TBA
16:30	Christina Thiele Technical University of Darmstadt, Germany Recent advances in orienting organic compounds for RDC structural analysis
17:15	**Poster session**, even-numbered posters
19:30	*Dinner*

Saturday, February 28

07:30	*Breakfast*
	Chairperson: Christian Griesinger
8:30	Jozef Kowalewski Stockholm University, Sweden NMR in paramagnetic systems in solution
9:15	Bert Heise Spin-Doc, Germany Structural studies of metal-organic frameworks by solid-state NMR spectroscopy and first-principles calculations
9:45	Zoltán Béni Gedeon Richter Plc., Hungary CASE: computer assisted structure elucidation
10:15	*Coffee break*
	Chairperson: Marek Potrzebowski
10:45	Anna Zawadzka-Kazimierczuk University of Warsaw, Poland High-dimensional ^{13}C-detected experiments for assignment of intrinsically disordered proteins
11:15	Ivana Biljan Slovenian NMR Center, National Institute of Chemistry, Slovenia Structural insights into disease-associated human prion protein mutants by NMR
11:45	Saurabh Saxena University of Warsaw, Poland High-dimensional NMR experiment for sequential assignment in ^{13}C labeled RNAs
12:00	Radek Pohl Institute of Chemical Technology, Czech Republic Probing the conformation of (1⟶2)-C-disaccharides by NMR
12:15	*Lunch*
14:00	*Free time*
19:30	*Dinner*

Sunday, March 1	
07:30	*Breakfast*
	Chairperson: Wiktor Koźmiński
9:00	Krzysztof Kazimierczuk University of Warsaw, Poland Sparsity in NMR and around
9:45	Vladimír Mlynárik Medical University of Vienna, Austria Chemical exchange saturation transfer (CEST) MR imaging
10:15	*Coffee break*
	Chairperson: Wiktor Koźmiński
10:45	Władysław P. Węglarz Institute of Nuclear Physics, PAS, Poland Can we do mouse brain histology *in vivo* using MRI?
11:15	Mateusz Urbańczyk University of Warsaw, Poland Non-uniform sampling meets DOSY
11:30	Poster prize winner
11:45	Closing
12:15	*Lunch*
14:00	*Departure*

Oral Presentations

On the human aspects of scientific thinking in NMR spectroscopy

Csaba Szantay, Jr.

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In this somewhat philosophical, and therefore admittedly off-the-wall presentation I will outline a conscious way of thinking, or an attitude, if you will, that our team has been cultivating for a while in our research facility, and which has proved to be highly useful not only in our everyday professional life, but also in private life. The main theme of this attitude is to develop a keen mindfulness of how our human nature influences our thoughts in science, and on how this influence can secretly lead even the smartest and most knowledgeable scientists into what we call "Mental Traps", resulting in cognitive errors ranging from widely held scientific misconceptions to faulty deductions in structure elucidation. These Mental Traps are an intrinsic feature of how we, as humans, think both in science and in our everyday lives, and by understanding and analyzing their nature, one can develop the enlightening faculty of detecting and avoiding them both in one's own and others' thoughts.

The topic was inspired by some conspicuous and long-standing misconceptions in the basic descriptions of the physics of NMR, and also by the pressing need never to misidentify a molecular structure in a competitive pharmaceutical R&D environment. In this largely non-technical presentation I will discuss several Mental Traps, mentioning some examples from both the theory and application of NMR. The presentation is also closely related to two other talks given by others in this conference, one on the quantum and classical aspects of basic NMR (Lars Hanson), and the other on computer-assisted structure elucidation (Zoltán Béni). The full version of this material is expected to be published in book form this year.

New methods based on ^{13}C direct detection to study intrinsically disordered proteins

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Recent progress in NMR instrumentation, in parallel to the growing interest in understanding the functional role of protein intrinsic disorder and flexibility, have stimulated the development of a variety of new NMR methods to study intrinsically disordered proteins (IDPs). The high flexibility and largely solvent exposed backbone typical of IDPs influence NMR parameters causing reduced chemical shift dispersion and extensive broadening of amide proton resonances, in particular approaching physiological conditions. These constitute general features of IDPs that need to be taken into account in the design of NMR experimental methods. ^{13}C detected NMR experiments now offer a valuable tool to address these peculiar features of IDPs. The experimental variants to improve the performance of ^{13}C detected NMR experiments to study IDPs will be discussed [1–6]. These open the way to characterize IDPs of increasing size and complexity and to in-cell studies. Several examples will be presented.

Acknowledgments: this work has been supported in part by the EC Projects IDPbyNMR (Contract no. 264257), INSTRUCT (contract no. 211252) and BioNMR (contract no. 261863).

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Fast-pulsing NMR techniques: general concepts, practical aspects, and selected applications

Bernhard Brutscher

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During the last decade, we have developed a series of fast-pulsing, multidimensional NMR techniques for the study of biological macromolecules. These experiments, namely SOFAST-HMQC, BEST-HSQC, and BEST-TROSY provide highest possible sensitivity in short experimental time by enhancing the steady-state ^{1}H, and eventually also heteronuclear spin polarization. As a consequence, a simple "BEST- optimization" of a particular pulse scheme may lead to a significant gain in experimental sensitivity, and allows reducing the overall time required for data collection. Fast-pulsing techniques also open up new possibilities for the NMR investigation of short-lived, or only transiently populated molecular states, as they allow the recording of higher dimensional (2D, 3D) NMR spectra during the short lifetime.

In this lecture, I will present the basic concepts of SOFAST and BEST techniques, their experimental implementation, and discuss the potential benefits for selected applications. In addition, I will show an application of these techniques to investigate at atomic resolution the structural and dynamic features of the major folding intermediate of the amyloidogenic protein β_2-microglobulin with a half-life of only 20 minutes.

Direct monitoring of correlated ensemble fluctuations in intrinsically disordered proteins

Dennis Kurzbach, Gerald Platzer, Thomas C. Schwarz, Agathe Vanas, and Robert Konrat

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Here we present a novel methodology, NMR-based paramagnetic relaxation interference (PRI), that allows for the first time to directly observe concerted motions and cooperatively folded sub-states in IDPs. The problem of experimental conformational averaging is circumvented by manipulation of electron relaxation rates in doubly spin-labeled proteins leading to a reduction of the paramagnetic effect due to spatial proximity of an NMR-observed nucleus and the two spin labels. A statistical analysis of PRI data yields a graphical display of compact conformations and correlated fluctuations in the conformational ensemble of the IDP Osteopontin.

With this method we provide mechanistic insight into the subtleties of conformational averaging in IDPs under changing environmental conditions and the relevance of correlated structural fluctuations of individual sub-domains in substrate binding of IDPs.

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MAP1B light chain and its interaction with microtubules

Markus Huber,[a] *Thomas Schwarz*,[b] Zsuzsanna Orban-Nemeth,[b] Morkos Henen,[b] Wiktor Koźmiński,[c] Szymon Żerko,[c] Saurabh Saxena,[c] Jan Stanek,[c] Leonhard Geist,[b] Friedrich Propst,[a] and Robert Konrat[b]

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Microtubule-associated protein MAP1B is a neuronal protein which has been shown to be essential for the maturation of synapses and network formation in murine brain development. Deregulation of MAP1B has been associated with several neuronal diseases. MAP1B can bind to microtubules as well as to F-actin and, thus, has been postulated to be responsible for coupling these two components of the cytoskeleton. MAP1B has been demonstrated to utilize its light chain for microtubule binding, but insights into its function are limited.

Here we report our efforts to understand the structure of the MAP1B light chain and its mode of interaction with microtubules. A light chain fragment containing the NH_2-terminal domain of the MAP1B light chain and was expressed in *E. coli*. We used 5D and 4D pulse sequences and sparse multidimensional Fourier transformation protocols in order to gain backbone as well as side-chain assignments. The assignment obtained indicated only a slight tendency towards alpha-helical conformations at the NH_2 terminus of the protein. A ^{15}N labeled sample was used to measure electron paramagnetic resonance (PRE) data on the construct. Here we gained information about a slight compaction of the protein at its very NH_2 terminus. Both of these observations agree with Meta Structure derived results on the protein's compactness. First investigations into the binding mode of MAP1B indicate that the protein contacts microtubules with its NH_2 terminus, and is capable to interact both with polymerized microtubules as well as with tubulin dimers.

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Simplifying proton-detected NMR spectra by spatially-selective excitation

N. Helge Meyer, Simon Glanzer, Nina Gubensäk, and *Klaus Zangger*

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Protons are the most often used nuclei for NMR structure elucidation of organic and biological molecules. Compared to other NMR detectable nuclei, ^{1}H spectra typically suffer from low resolution and severe signal overlap, mainly due to extensive scalar coupling between protons. Homonuclear broadband decoupling (pure shift NMR), which leads to a collapse of ^{1}H signals into singlets, vastly increases the resolution, which in some cases corresponds to a theoretical signal dispersion of NMR spectrometers at several GHz [1]. One of the approaches for homonuclear broadband decoupling in the indirect dimension of two- and multidimensional NMR spectra uses frequency-selective pulses during a weak gradient field [2].

We recently reported an adaption of this method to achieve homonuclear broadband decoupling during acquisition [3]. Scalar coupling information, which is often key in analyzing chemical structures, is of course completely lost in such experiments. Two methods, which constitute a compromise between pure-shift spectra and fully coupled spectra will also be presented: real-time SERF spectra [4] and real-time J-scaled proton spectra. With the first of these, 1D spectra are obtained which contain scalar coupling to one selected signal only, while J-scaling allows the recording of proton spectra with reduced coupling constants, reminiscent of off-resonance heteronuclear decoupling.

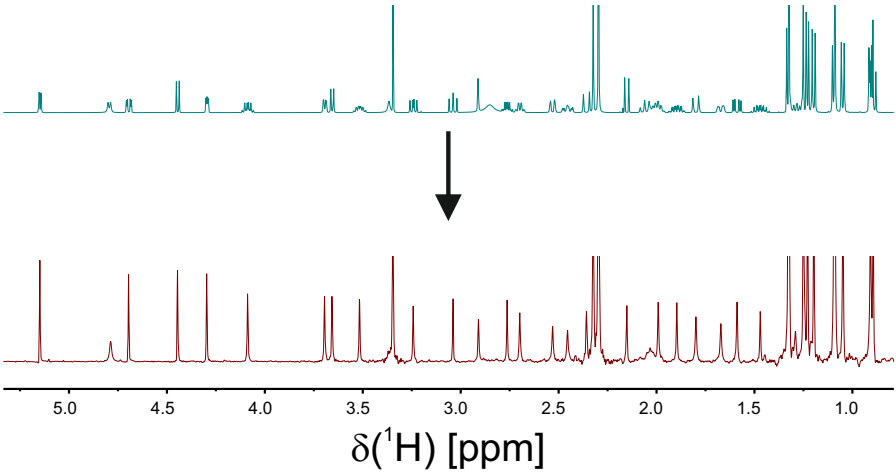

Figure 1: Regular and real-time pure-shift spectra of azithromycin

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Precise measurement of heteronuclear coupling constants: novel applications of broadband proton-proton decoupling

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A variety of novel methods have been developed in recent years that reduce the complexity of ^{1}H NMR spectra by collapsing the multiplet structure of each resonance into a singlet. These experiments deliver pure chemical shift information without the complication of homonuclear coupling interactions and with significantly increased resolution in the direct proton dimension.

Implementing this broadband proton-proton decoupling methodology in the CLIP/CLAP-HSQC [1] and HSQMBC [2] experiments the undesired proton-proton splittings are removed from the heteronuclear multiplets, thus the heteronuclear couplings of interest can be determined simply by measuring the frequency differences between the peak maxima of pure doublets.

The potential of these experiments will be demonstrated on molecules featuring extended networks of mutually coupled protons. Additional multiplet fitting procedures would normally be required to extract long-range heteronuclear coupling constants when using standard HSQMBC experiments for such cases. Incorporation of the recent PSYCHE [3] pulse sequence element or of instant (real-time) homonuclear broadband decoupling [4] methodology in the HSQMBC sequence, resulting in significant improvements in sensitivity, will also be presented.

Financial support from OTKA K 105459, OTKA NN 109671 and TÁMOP-4.2.2/A-11/1/KONV-2012-0025 is gratefully acknowledged.

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Visualisation of basic NMR: quantum and classical aspects

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Handwaving and semi-classical graphics are widely used to illustrate spin dynamics such as excitation and echoes. Quantum descriptions are often accompanied by different figures such as level diagrams and cone figures. In this talk, the relation between quantum and classical mechanics is discussed with a particular focus on visualisation. It is argued that the classical and the quantum descriptions of basic NMR are more similar than they may first appear, and that "classical" illustrations can accurately depict essential aspects of quantum NMR [1]. In contrast, illustrations meant to convey quantum mechanics often do not [2]. Figures that are misleading or divert attention from crucial aspects are abundant. Simple graphical tools aimed at early NMR and MRI education are demonstrated.

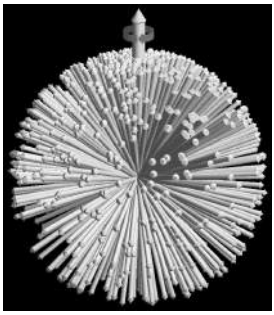

Figure 1: Bloch vector vizualization [3] of thermal equilibrium which is equally valid from classical and quantum perspectives.

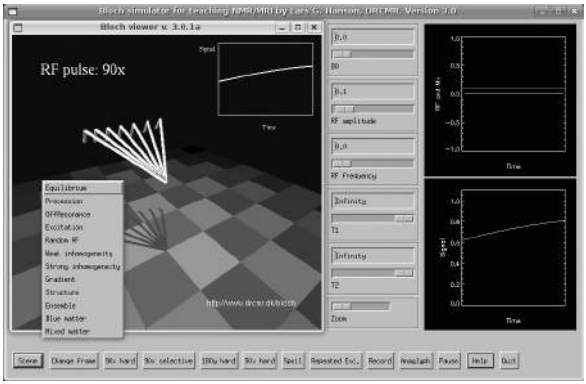

Figure 2: The Bloch Simulator [4] is a free Flash™application running directly in most browsers. It can be used to explore basic NMR and MRI methods such as echo formation.

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Application of AQARI (Accurate Quantitative NMR with Internal Substance) to quality control of organic reagents

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Quantitative HPLC (high performance liquid chromatography) analysis is typically carried out by using a reference substance which is the same as the compound to quantify. However, certified reference substances are sometimes unavailable due to strict requirements and high cost of their production. Therefore, commercial reagents are often used as reference substances in chromatographic assays, even though their absolute purity is unknown. To ensure the reliability of quantitative assay, absolute purity is crucial. qNMR (quantitative NMR) is known as one of absolute quantification methods suitable for purity determination of organic compounds [1]. Recently, ^{1}H qNMR has drawn much attention in many fields as it provides accurate and precise quantitative values without the usage of a reference compound that is chemically identical to the analyte. In addition, qNMR analysis with SI (Le Système International d'Unités, International System of Units) traceability is possible by using appropriate protocol. Therefore qNMR qualifies as a method to evaluate reference substances for other analytical methods, such as HPLC. qNMR has already been employed for purity determination in Japanese regulatory standards such as Japanese Pharmacopoeia [2] and Japan Specification and Standard for Food Additives [3, 4].

In this study, we have developed a system for quality control of organic reagents by 1H qNMR. In particular, we have used AQARI (Accurate quantitative NMR with internal substance) [5], which is one of qNMR methods and provides highest precision and accuracy than other qNMR methods. The protocol for AQARI includes not only NMR measurement but also sample preparation. We have evaluated a large variety of reagents and here we discuss feasibility of AQARI for purity determination. We have found that AQARI is able to determine the purity of reagents with accuracy of approximately 1% and can be applied as an efficient quality control of organic reagents. By using reference substances with SI traceability and incorporating purity obtained by qNMR into calculation of quantitative values in quantitative analyses by HPLC and other methods, SI traceability is ensured. Therefore, our approach significantly improves reliability of commonly used quantitative methods.

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New point of view in utilising tags for structural analysis of complex mixtures

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Even the most powerful multidimensional (nD) NMR methodologies alone cannot solve structures of compounds contained in mixtures of thousands of small molecules. Some form of "spectroscopic separation" is therefore required. To achieve this, we have developed isotope-filtered nD NMR spectroscopy [1] that allows structural investigation of isotopically tagged molecules in complex, inseparable mixtures.

Incorporating isotopically labelled moieties within targeted functional groups of small organic molecules opens a unique possibility for structure characterisation of molecules in complex mixtures. Although isotopic tagging has been used in the analysis of mixtures in the past, we propose a new paradigm. Rather than focusing on the chemical shifts of the tags only, we use the tags for accessing chemical shifts and coupling constants of the parent molecules. We illustrate this approach by ^{13}C-methylation of hydroxyl and carboxyl groups in combination with purpose-designed nD NMR experiments.

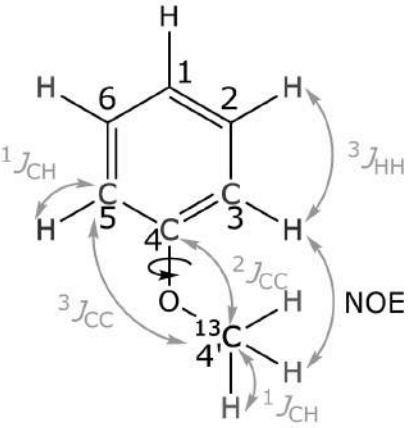

Figure 1: Polarisation transfer scheme

Scheme on the right shows the interactions that can be used to transfer the polarisation in a ^{13}C-methylated phenol. These couplings were utilised in the design of nD NMR experiments that provide multiple correlated chemical shifts and coupling constants of aromatic rings nuclei. In these experiments, the signals from unlabelled molecules are eliminated, providing much needed simplification of NMR spectra of complex mixtures. The obtained information enabled the structures of derivatised compounds to be solved.

The developed methodology is aimed at the analysis of the aromatic moieties of humic substances (HS), the main organic component of soil and amongst the most complex mixtures on Earth. We have recently applied it to characterise major substitution patterns of phenolic moieties of fulvic acid isolated from a Scottish peaty soil [2].

Our approach is not limited to studies of HS, neither is the methylation as the way of introducing labels to report on the parent molecules. We are currently exploring several different approaches based on the principles outlined here.

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From monosaccharides to polysaccharides: NMR applications

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Carbohydrates, or saccharides, are the most abundant of the four major classes of biomolecules, along with proteins, nucleotides, and lipids. There are various types of saccharides, including mono-, oligo- and polysaccharides [1]. Traditionally, NMR spectroscopy has been one of the major tools to foster the advance of carbohydrate chemistry [2]. The study of the structure, conformation and dynamics of mono- and oligosaccharides is important because of their biological relevance. The high solubility and their generally good NMR properties, resulting from relatively small molecular sizes, narrow lines and sufficiently slow relaxation allow thorough investigation of a great variety of individual carbohydrates by relatively simple 1D and 2D NMR methods.

Recent results from the application of this knowledge and questions arising when studying problems like chemical and metabolic profiling, carbohydrate complex formation and stucture of polysachharides will be presented and discussed.

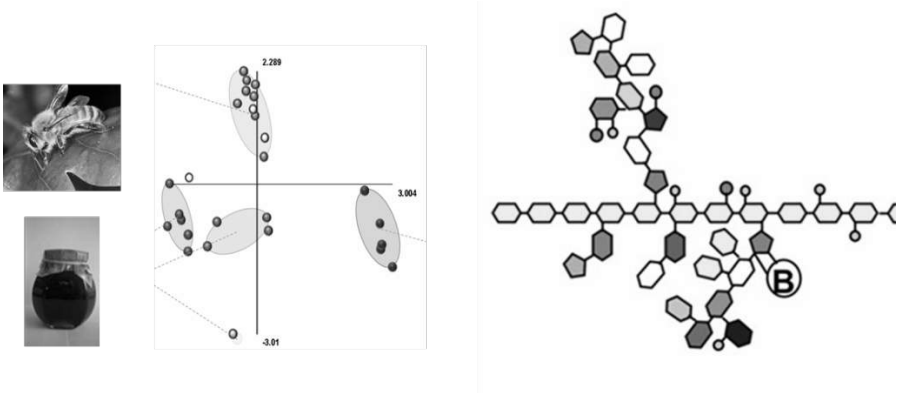

Figure 1: From chemical profiling to polysaccharide structure

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NMR spectroscopy in structural biology and for drug validation and development in neurodegeneration

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An introduction will be given into the scope of NMR spectroscopy to address questions in natural product chemistry [1–19], membrane proteins [20–24] and signalling. Then a more applied project in the department will be discussed where small molecules play a decisive role in redirecting aggregation. Parkinson's disease features aggregates of α-synuclein, so called Lewy bodies, which are connected to neuronal dysfunction and death. With NMR in liquid and solid state, we have characterized the polymorphic forms of α-synuclein [25–31]: a) Monomers, that are considered to be the healthy form, are so called intrinsically disordered proteins lacking secondary structure but having partial tertiary structure. The latter autoinhibits aggregation. In line with this we observe that mutants that destabilize the partially folded form aggregate faster and exhibit higher toxicity in animal models. b) Oligomers of rather well defined size are observed that have less β-structure than the fibrils. They are on-pathway to the fibrils. c) Fibrils are formed which could be characterized structurally by solid state NMR and are non-toxic. From the structure mutants have been predicted that don't form fibrils but oligomers at higher concentration. They have dramatically increased toxicity in animal models.

The "aggregation landscape" can also be modified by small molecules [32–34]. With screening and targeted libraries we identified compounds that reduce the size of oligomers *in vitro* and *in vivo*. They are off-pathway, since no fibrils are formed any more. Biophysically they prevent pore formation in membranes, an activity of aggregates that is discussed as a reason for toxicity. Oral application of these highly bioavailable compounds to various animal models (*C. elegans*, fly, mouse) of PD rescues the phenotype, i.e. reduces the aggregates and leads to an improvement of the behavioural phenotype with respect to locomotion in PD, learning and memory in AD and survival in CJD as well as PD and AD. Interestingly, the *in vitro* biophysical characteristics found first for α-synuclein are also observed for proteins responsible for other diseases, namely Creutzfeld-Jacob and Alzheimer's disease.

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Investigating dynamic layers of cellular information transfer

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In this lecture, I will discuss two examples where information transfer during transcription and translation is dynamically regulated. The first example deals with riboswitches. Riboswitches are gene regulatory elements located in the 5'-untranslated regions of messenger RNA (mRNA). Ligand binding to an aptamer domain of riboswitches induces either the up- or down-regulation of the expression of ligand-associated genes. Riboswitch regulate gene expression either at the level of transcription or translation. For transcriptional riboswitches, ligand binding supposedly induces a conformational switch between mutually exclusive antiterminator or terminator conformations that represent the on- and off-states of the switches. We show that in stark contrast to this classical model for riboswitch function three different transcriptional riboswitches adopt the terminator conformation (off-state) at thermodynamic equilibrium regardless of the presence or absence of the ligand. By investigating the guanine- and hypoxanthine-sensing *xpt-pbuX* riboswitch from *Bacillus subtilis*, we find that in contrast to the full-length mRNA, transcription intermediates undergo ligand-dependent conformational changes. Importantly, in the absence of ligand, the RNA was able to adopt the antiterminator conformation in a transcription intermediate that was kinetically trapped and did not refold fast enough to form the longer and more stable terminator conformation in the limited time window available during transcription. This kinetic trapping allows the RNA-polymerase to escape from the termination site before the terminator is folded and to maintain gene expression in the absence of ligand. In the presence of ligand, early ligand binding stabilised the aptamer domain, suppressed the formation of the antiterminator conformation, and, thus, accelerated formation of the terminator conformation by at least two orders of magnitude, leading to gene repression. Transcriptional regulation therefore executes the genetic decision during a single-round of transcription and is critically dependent on the kinetics of ligand binding and RNA refolding that constantly change as the length of the mRNA chain grows during transcription.

The second example deals with protein folding and reports that differential usage of synonymous codons governs co-translational folding and final protein structure. The genetic code is degenerate, with up to six synonymous codons encoding a given amino acid in the protein. The occurrence of synonymous codons in open reading frames (ORFs) of genes is not random, suggesting the existence of evolutionary constraints on codon choice.

In this lecture I will present data demonstrating that synonymous codon usage governs the kinetics of translation, co-and post-translational protein folding and final protein structure. The ribosome-bound nascent chains of the mammalian eye lens protein, gamma-B crystallin, expressed from two gene variants with different synonymous codon composition but encoding the same polypeptide, attain different conformations as indicated by altered in vivo stability and in vitro protease resistance. Our 2D NMR spectroscopic data suggest that the observed structural differences are associated with different cysteine oxidation states of the synonymous variants. Synonymous codon usage was found to alter local and global rates of translation and affect the efficiency of co-translational folding of protein domains as well as the ultimate stable conformation attained by the protein.

Pitfalls in RNA structure prediction

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RNA molecules play important roles in the regulation of gene expression at various levels. For example, RNAs are active participants in the regulation of gene expression by interference or by riboswitches, in the processing of RNA introns and in protein synthesis by the ribosome. Knowledge of the three-dimensional structures of these molecules is a fundamental prerequisite to a complete understanding of RNA function.

New RNAs are being discovered much faster than their three-dimensional structures. Experimental determination of sequences of gens and entire genoms, from which the sequences of RNA can be reliably inferred is much cheaper and faster than experimental determination of the structures. In a consequence, the disparity is increasing between known RNA 3D structures and known RNA sequences.

To provide insight into RNA function and to guide experimental work in biology and biochemistry several strategies has been identified, which now allow for reasonably accurate prediction of 2D and 3D structures. However, it is important to remember that the prediction of any RNA structure is just an approximation based on a number of simplifying assumptions. Many RNAs contain both structured and functionally important but flexible elements. Structure may also change because of environmental factors, e.g. binding of other molecules or the composition of the solution (salt, pH). At present, the prediction may be close to a structure found in nature or very far off, depending on the length and nucleotide sequence of an RNA molecule.

The advantage of NMR spectroscopy in studying RNA is an ability to monitor its structure and dynamics in physiologically relevant solution. Several examples of RNA molecules for which NMR spectroscopy has provided structural information inaccessible to the other methods will be presented.

Structural diversity of guanine rich sequences in genomes of human papillomaviruses

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Human papillomaviruses (HPV) are a diverse group of viruses that are important human pathogens. Infection with certain low-risk types of HPV can lead to formation of warts and lesions, while infection with high-risk HPV can cause cancerous changes of head and neck, skin and anogenital area. Among those, cervical cancer represents one of the most common cancers worldwide and preceding infection with high-risk HPV is required for cancer development. Guanine-rich sequences in the genomes of HPV have potential to fold into four-stranded DNA structures or G-quadruplexes that were shown to act as controllers of processes of replication, transcription and translation. G-quadruplex stabilization with ligands could therefore make it possible to control HPV replication and/or gene expression.

We have analyzed G-quadruplex forming potential of guanine-rich sequences found in different HPV genomes in the presence of K+ with help of electrophoresis, CD, UV and NMR spectroscopy [1]. Most of them have been shown to fold into G-quadruplexes with polymorphic behaviour. Location of the sequences in regulatory long control region and in the regions coding for early (E1 and E4) and late (L2) proteins suggests a potential role in transcription, replication and alternative splicing. Our results represent a starting point for the design of specific ligands for viral G-quadruplex motifs and suggest yet unexploited pathways for the control of viral replication and transcription.

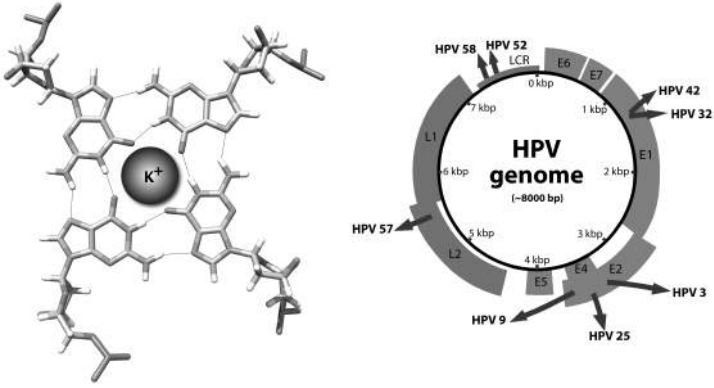

Figure 1: G-quartet, building block of G-quadruplexes (left) and locations of G-rich sequences in HPV genomes (right).

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A tetrahelical DNA fold not stabilized by G-quartets

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DNA is known to form diverse double-, triple- and tetrahelical high order structures whose formation is highly susceptible to nucleotide sequence and surrounding conditions. Guanine and cytosine rich sequences are known to form G-quadruplexes and i-motifs respectively [1]. G-quadruplexes have been shown to exist in vivo and their formation has been implicated in essential biological processes. The basic building blocks of G-quadruplexes are G-quartets, planar arrangements of four Hoogsteen-type H-bonded guanine residues that are stabilized by Na^+, K^+, NH_4^+ or other mono- or dications, but cannot be stabilized by Li^+ ions. Guanine residues involved in G-quadruplex core can be connected by diagonal, edgewise and propeller loop regions which can also include adenine, thymine and cytosine residues. Outside the G-quadruplex core, residues can protrude into solution or interact with other residues and form H-bonds that are not limited to Watson-Crick geometries. GC base pairs have been known to form GCGC-quartets exhibiting different H-bonding geometries which are highly susceptible to the surrounding conditions such as presence of different cations [2, 3].

Here we show that guanine rich DNA sequences with GGGAGCG repeats found in the regulatory region of the PLEKHG3 gene are capable of forming tetrahelical DNA structures that are distinct from G-quadruplexes [4]. No G- or GCGC- quartets are present in the fold and the overall topology is conserved in the presence of Li^+, Na^+, K^+ and NH_4^+ ions. The structure contains many unique structural features and is stabilized by four G-C, four G·A and six G·G base pairs in N1-carbonyl symmetric geometry.

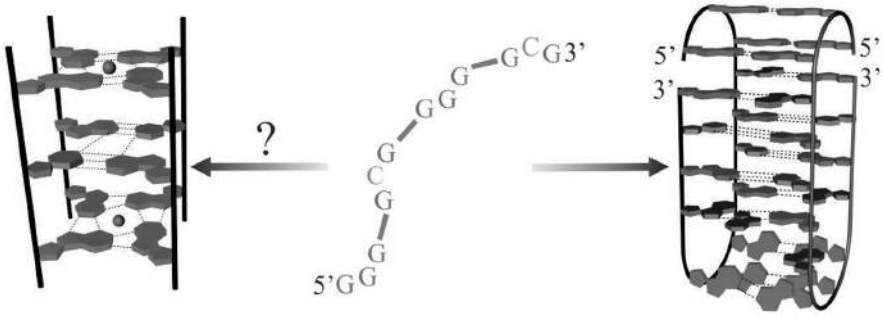

Figure 1: The expected (left) and actual (right) topology adopted by GGGAGCG repeats

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Structural insights into aberrant splicing of CFTR exon 9

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Alternative pre-mRNA splicing processes play a key role in creating the vast number of gene products underlying our complex organism. The processing of pre-mRNAs is tightly regulated and any imbalance can change the outcome of gene expression, often leading to disease. Despite the importance of alternative splicing regulation, our knowledge at the molecular level is still in its infancy.

We study the regulatory RNA-protein and protein-protein interaction networks surrounding the aberrantly spliced cystic fibrosis transmembrane conductance regulator (CFTR) exon 9. Skipping of this exon 9 leads to the production of a non-functional chloride channel, which is associated with severe forms of cystic fibrosis. This unfavourable splicing event depends primarily on TDP-43 (43 kDa TAR DNA binding protein) which binds to a UG-rich region upstream of the 3' splice site (ss) of exon 9 and this, in concert with other splicing factors, prevents the recognition of the 3'ss of exon 9. Thus exon 9 is excluded from the spliced mRNA and this results in a non-functional protein product.

As a first step towards understanding the molecular basis of aberrant CFTR exon 9 splicing, we solved the solution structure of TDP-43 RRMs in complex with UG-rich RNA. Ten nucleotides are bound between both RRMs with six being sequence-specifically recognized. Among those, a central guanosine is found interacting with both RRMs and stabilizing a novel tandem RRM arrangement. Mutations which eliminate recognition of this key nucleotide or crucial inter-RRM interactions disrupt RNA binding and TDP-43 - dependent splicing regulation. In contrast, point mutations that affect base-specific recognition in each RRM have weaker effects. Our findings reveal not only how TDP-43 recognizes UG repeats but also how RNA-binding dependent inter-RRM interactions are crucial for TDP-43 function.

Currently, we are reconstituting the entire aberrant 3'ss complex and we are determining the structure of two TDP-43 copies bound to the 3'ss RNA, a 50 kDa RNA-protein complex, using various selective isotope labelling approaches. Novel molecular insights into this regulatory RNA-protein and protein-protein interaction network emerging from SAXS experiments and NMR spectroscopy as well as challenges working on large RNA-protein complexes will be discussed.

Application of homonuclear mixing on ^{1}H in 100 kHz magic angle spinning

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^{1}H NMR of rigid samples becomes a major choice thanks to the inventions of ultrafast magic angle spinning (MAS), which now provides frequencies over 110 kHz [1, 2]. In this paper, we will introduce recent progress of 1H-1H magnetization mixing at ultrafast MAS rate [3–7]. Although rapid spin diffusion due to strong ^{1}H-^{1}H homonuclear dipolar interaction quickly mix the longitudinal ^{1}H magnetization at static or moderate MAS rate (< 20 kHz), spin diffusion process becomes very slow due to the suppression of ^{1}H-^{1}H dipolar interactions under the fast MAS regime. Thus it is needed to re-introduce ^{1}H-^{1}H dipolar interactions for homonuclear correlations, homogenizing ^{1}H magnetizations, transferring ^{1}H magnetizations, etc.

First, we focused on the radio frequency driven dipolar recoupling (RFDR) sequence which is widely used to re-introduce homonuclear interactions in zero-quantum form mostly for ^{13}C [8]. We experimentally and numerically investigated the phase cycling in RFDR for ^{1}H-^{1}H mixing. While XY814 is known as the best for ^{13}C [9], XY414 is the best choice of phase cycling because of robustness towards r.f. field inhomogeneities and chemical shift offset, if the r.f. field strength is strong enough (> 400 kHz).

Secondly, we applied RFDR to reduce the repetition delay, thus shorten the measurement time. ^{1}H T_1 relaxation time is uniform in rigid-solid samples due to rapid spin diffusion at static or moderate MAS conditions. However, at fast MAS rate, each ^{1}H nucleus tends to show individual ^{1}H T_1 relaxation time due to suppression of ^{1}H-^{1}H spin diffusion [10]. This is quite usual for most low gamma nuclei like ^{13}C, ^{29}Si because of absence of spin diffusion. In that case, we need to wait long enough time to allow the nuclei of interest to recover to the thermal magnetization, even if the other nuclei are already recovered. Fortunately, in ^{1}H system at ultrafast MAS, we can reintroduce ^{1}H-^{1}H spin diffusion by applying RFDR and bring the magnetization from ^{1}H with short T_1 to the other ^{1}H with long T_1. This can significantly reduce the repetition delay, leading to time-reduction factor of 10 in the favorable case.

Thirdly, RFDR is utilized to correlate between ^{15}N through ^{1}H-^{1}H mixing. Since the homonuclear ^{15}N-^{15}N interactions are quite weak, it is not straightforward to achieve ^{15}N-^{15}N correlations [11]. Here we transfer ^{15}N magnetization via ^{1}H-^{1}H spin diffusion by RFDR to achieve ^{15}N-^{15}N correlation and observe signal ^{1}H detection. This involves four time CP transfer between ^{1}H and ^{15}N, however, due to high CP efficiency and high sensitivity of ^{1}H indirect detection, ^{15}N-^{15}N correlation is successfully measured.

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2D and 3D CP-VC as tools for dynamics study

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One of the biggest achievements of modern solid state NMR spectroscopy is its ability to determine accurate inter-nuclear distances, which can be afterward used as structural restraints for reconstruction of three-dimensional structures of the condensed matter. The most common strategy is based on the analysis of homo- and/or hetero-nuclear dipolar couplings, which are both inverse proportional to the cube of inter-nuclear distances. Among different interactions, C—H and N—H one-bond contacts are of great interest in the context of characterizing inter-molecular arrangement via hydrogen bonding as well as backbone and side-chain dynamics in biological molecules. Indeed, the partial averaging of C—H and/or N—H dipolar couplings gives information about the geometry and amplitude of the motional processes in the solid state.

During the last decades, different methodological approaches, both for static samples and samples under magic angle spinning (MAS), have been introduced in order to improve the quality and reliability of obtained data. The big achievement in the field of measurements of X—^{1}H dipolar couplings was the introduction of the PISEMA technique and its different variants, which allowed the determination of dipolar interactions under MAS, e.g. PILGRIM. Two years ago we demonstrated that a very simple experiment, **c**ross-**p**olarization with variable **c**ontact time (CP-VC) [1], is very efficient under ultra-fast MAS (ν_{RO} = 60 kHz) to measure accurately the C—H and N—H distances, and to analyze the dynamics of bio-molecules.

Very recently we developed new multidimensional solid-state NMR methodology, which permits the analysis of ^{1}H-^{13}C dipolar splittings in a simple and accurate way and further scrutiny of the molecular motions in side chains in nanocrystalline proteins. The power of the technique is demonstrated in 3D NMR CPVC-RFDR correlation experiments in two proteins, GB1 and DLC8. This presented methodology is general and can be extended to other systems.

In my presentation I will briefly present some methodology and possibilities of advanced solid state NMR. After that, I will discuss how to probe the dynamics of aromatic residues: phenylalanine, tyrosine and tryptophan using our new methodological approaches.

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Real time $\mathcal{J}$-scaling in nuclear magnetic resonance

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NMR spectroscopy is one of the most frequently used methods for structural studies of small to medium sized organic and biomolecules. Because of its high natural abundance, widespread occurrence and high sensitivity, ^{1}H nuclei are often used in this process. Resonance frequencies and scalar coupling constants can provide important structural information. However, due to the restricted chemical shift range of protons, the signals are often overlapped, rendering the extraction of structural information difficult or sometimes impossible. The application of pure-shift methods is one way to increase the resolution of ^{1}H NMR spectra [1–3], which yield singlet-only spectra, reminiscent of proton broadband decoupled ^{13}C NMR spectra.

While these experiments provide highest-resolution 1D proton NMR spectra, scalar coupling information, which is often key in analyzing chemical structures, is completely lost in such experiments. Several techniques have been described in order to simplify spectra (increase their resolution) and still allow the extraction of scalar coupling information. Most commonly two-dimensional E-COSY type spectra are employed, yielding multiplets where active and passive couplings are distinguished or 2D $\mathcal{J}$-resolved experiments, separating the chemical shift in the direct from $\mathcal{J}$-coupling information in the indirect dimension. For complicated multiplets or larger molecules, these spectra still often result in crowded multiplet patterns. The aim of this research is to provide a method which allows the extraction of homonuclear scalar coupling constants and chemical shifts at the same time with high resolution. While in some situations higher resolution is needed for chemical shifts, other cases require higher resolution in scalar coupling interactions. Therefore, it would be desirable to be able to continuously scale the ratio of chemical shift over scalar coupling constants, preferably in the detection dimension of the NMR spectrum.

Here we present a pulse sequence [4] which allows the real-time (single scan) or expansion scaling of homonuclear coupled multiplets by a user-defined factor, whereas the chemical shift values are left untouched. The down-scaling technique is based on a recently developed instant homonuclear decoupling method [2]. This experiment utilizes slice selective excitation, which can be achieved by selective pulses during a weak field gradient. The selective pulse hits all protons, but dependent on the position in the sample tube different signals are excited. By modifying this experiment, a total decoupling is replaced by a partial decoupling, dependent on the J-scaling factor λ. This method separates overlapping peaks without losing multiplicity.

On the other hand, in less crowded spectral regions up-scaling achieves a major resolution improvement for scalar couplings. Furthermore, it has the potential to reveal splittings that are "buried" under the spectral line width in regular spectra. For example, by increasing the coupling constant by a factor of 7 in *n*-propanol, it was possible to observe and measure a splitting difference of 0.7 Hz. This was not possible in the regular NMR spectrum, where the line width is on the order of 1.8 Hz. Additionally, the up-scaling sequence does not depend on slice-selective excitation and has therefore a sensitivity comparable to regular NMR spectra.

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Protein MAS DNP at 190 K and MAS triple-resonance spectroscopy of membrane proteins

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Solid-state NMR enables the investigation of heterogeneous, complex biological samples at high resolution. In future, a major factor facilitating such investigations will be dynamic nuclear polarisation (DNP), which was introduced to increase signal-to-noise by one or two orders of magnitude. During the DNP process, electron polarization is transferred to the surrounding 'core' nuclei, subsequently to the bulk nuclei, and then further on to the molecule of interest. This process depends on several factors, among them are the relaxation behavior of the electrons and protons in the sample. In order to improve the quality of DNP spectra and to obtain maximum signal-to-noise, new radicals were synthesized and employed in measurements of protein samples around 190K. Enhancements in the range of 15-20 were observed in this temperature range while acceptable spectral resolution is observed. In this context, mechanisms of polarisation propagation are analysed and schemes for optimization of samples will be presented. Application of DNP to protein systems will be shown and discussed.

In the second part of the talk, investigations of membrane proteins are presented which include extensive deuteration, and subsequent detection of protons under magic-angle spinning conditions. Various schemes for the usage of proton chemical shifts for achieving sequence-specific assignments will be discussed. These studies involve an ABC-Transporter that imports positively charged amino acids into bacteria and outer membrane protein G form e.coli, OmpG.

NMR crystallography

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NMR Crystallography is an idea which combines analysis of X-ray powder diffraction (PXRD) data, quantum mechanical calculations and NMR measurements. The key technique in this strategy is solid state. SS NMR provides rich set of constraints, which are extremely useful in structural analysis of condensed matter. First, spectra provide a "fingerprint" of the local structure and represent the local electronic environment for each nucleus under investigation. NMR responds to the short-range environment of relevant atoms and is not directly influenced by long-range order. The most important NMR parameter, chemical shift, gives information about intermolecular interactions. Analysis of principal elements of the chemical shift tensor δ_{ii} provides detailed information about the electronic distribution around each individual nucleus. Inter- and intra-molecular hydrogen bond linkages can be identified. Information on crystallographic asymmetric units is especially readily available, usually merely by counting lines. Polymorphs are usually easily distinguished. Phase transitions can be monitored. Crystallographic disorder is detectable, and distinctions between spatial and temporal disorder can be made. Measurement of dipolar coupling constants yields through-space inter-atomic (i.e. internuclear) distances, though these will be modulated by local mobility.

During the talk, a few applications of NMR crystallography approach will be presented. In particular, attention will be paid to the following problems:

1. Fine refinement of structure of material after thermal or chemical transformations when single crystal is not available [1].
2. Refinement of structure when the deposited X-ray data are poor quality [2, 3].
3. Fine refinement of structure when only X-ray data of powder are available (*de novo* refinement) [4].
4. Search for new crystallographic forms, e.g. polymorphs, host-guest complexes, and so on [5].

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Structural studies of metal-organic frameworks by solid-state NMR spectroscopy and first-principles calculations

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Solid-state NMR spectroscopy has gained significant power as a tool for structural analysis of crystalline lattices since it became possible to predict NMR chemical shifts ab-initio using the density-functional theory (DFT) with the gauge-including projector-augmented wave (GIPAW) approach. The class of materials that benefitted the most from these advances is probably the class of crystalline pharmaceuticals, for which ^{1}H and ^{13}C chemical shifts can be predicted very accurately. For such materials the development of the ab-initio chemical shift prediction software even lead to attempts of NMR crystallography, i.e., to the structure-determination approach in which for a material with an unknown structure the quality of trial structural model is evaluated by the comparison of the measured and calculated chemical shifts.

Metal-organic framework (MOF) structures can also be studied by NMR, because both major building units of these hybrid materials, organic linkers and metal-oxo clusters, contain NMR-active nuclei. In this contribution we will show on a series of magnesium-based MOFs that the combination of solid-state NMR spectroscopy and first-principles calculations provides valuable information about the structure of MOFs, eventhough these materials are generally much more complex than the small-molecule pharmaceutical substances. We will show that ^{13}C chemical shifts and ^{25}Mg quadrupolar coupling constants calculated by the DFT/GIPAW and DFT/PAW approaches are very sensitive to the structural models, so that the comparison of the calculated and the measured values can often tell whether the diffraction-based structural models are correct or not.

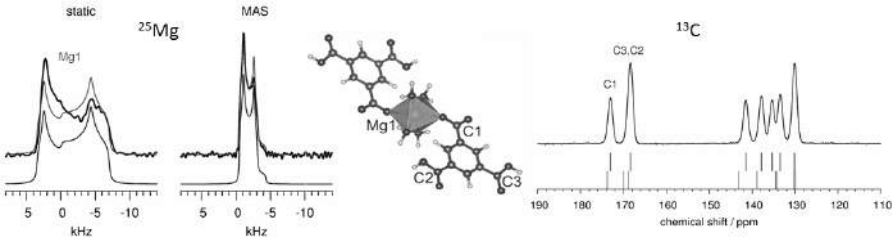

Figure 1: ^{25}Mg and ^{13}C solid-state NMR spectra of Mg-based MOF

A particular advantage of NMR spectroscopy over diffraction techniques (or its complementarity to these techniques) is its ability for inspecting motifs that do not exhibit long-range order. In MOFs such motifs are often related to guest molecules located within the pores and also to various linker molecules distributed within the frameworks of the so-called mixed-linker or multivariate MOFs. Using mixed-linker DUT-5 MOF we shall demonstrate that relatively simple NMR techniques, accompanied by numerical simulations and first principles calculations, can provide valuable information about the incorporation and distribution of different linker molecules within the framework, i.e. they can provide information that is usually not accessible to diffraction techniques.

Recent advances in orienting organic compounds for RDC structural analysis

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Residual dipolar couplings (RDCs), which belong to the class of anisotropic NMR parameters, can yield information complementary to ^3J couplings and NOE parameters for the determination of the three dimensional structure of organic or organometallic compounds by high-resolution solution state NMR spectroscopy. A lot of progress in the development of new alignment media compatible with organic solvents has been made within the last 10 years [1].

This talk will focus on new columnar liquid crystals based on benzo-1,3,5-tricarboxamides [2] and chemically cross-linked PDMS gels [3, 4]. The latter even allowed the alignment and structure determination of an unstable palladium intermediate [5].

Acknowledgements: Funding by the ERC (starting grant no. 257041 to C.M.T.) and the German Research Council (research unit FOR 934) is gratefully acknowledged.

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NMR in paramagnetic systems in solution

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Paramagnetic systems contain unpaired electrons, which give rise to large magnetic moments. The electronic magnetic moment interacts with the magnetic moments of nuclear spins through the hyperfine interaction, further divided into dipolar and scalar parts. The hyperfine interaction has a profound effect on the NMR spectral properties (shifts, relaxation and splittings). A brief overview of the theory [1, 2] will be provided and illustrative examples, concerned with paramagnetic proteins [3], MRI contrast agents [4] and free radicals in solution [5], will be presented.

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And now to something completely different: Fast Field Cycling NMR Relaxometry

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Fast Field Cycling (FFC) NMR relaxometry [1, 2] offers a convenient way to measure longitudinal (T_1) relaxation constants over a large range of field strengths for all kinds of sample types. As T_1 relaxation is sensitive to molecular motions at the Larmor frequency, it can deliver insights into dynamic processes in the sample. Unlike with NMR spectrometers and conventional low-field relaxometers, FFC relaxometers measure T_1 relaxation not only at one fixed field strength but an entire nuclear magnetic relaxation dispersion (NMRD) profile can be obtained from a field strength of a few kHz up to tens of MHz. Hence, dynamic processes are accessible of in a large range of materials like polymers, metals, porous media, gels, food, biological and any liquid or solid sample in general – all of them having their sensitive area of molecular motion in a different frequency regime typically coverec by the relaxation profile that FFC can deliver.

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CASE: computer assisted structure elucidation

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The development of modern computer assisted structure elucidation (CASE) software tools receives much attention by the worldwide community of chemists and NMR spectroscopists. Whether artificial intelligence can eliminate human error, or even the need for human spectrum-interpretational knowledge, in structure elucidation is certainly an intriguing and important question in its own right, and is of particular interest within the conceptual and philosophical framework of Mental Traps, which will be introduced in some detail in another talk at this conference (Csaba Szántay).

In this presentation, after a short, non-technical introduction to CASE software modules, we will address the above question by examining the capabilities of an expert system through some real-life examples for structure elucidation problems where Mental Traps could be caught in the act.

High-dimensional ^{13}C-detected experiments for assignment of intrinsically disordered proteins

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High-dimensional ^{13}C-detected NMR experiments have proven useful for resonance assignment of intrinsically disordered proteins (IDPs). Acquired using non-uniform sampling [1], high-dimensional experiments provide spectra with extraordinary resolution. Carbon detection [2] facilitates assignment when proline residues are abundant in a given protein or when the amide protons undergo fast chemical exchange. Here we present three such novel experiments: 4D (HACA)CON(COCA)NCO, 5D HabCabCO(CA)NCO and 5D HNCO(CA)NCO.

Data processing is performed using sparse multidimensional Fourier transform (SMFT) [3], based on the concept of fixing some of spectral dimensions to the frequencies known from a so called basis spectrum, acquired in advance. The fixing protocol was in this study developed to so called multiple-fixing method, which facilitates access to spectral data. The experiments were tested on a 140-aa-long IDP, α-synuclein. Resonance assignment was performed automatically, using the TSAR program [4], yielding almost 93% of correctly assigned resonances.

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Structural insights into disease-associated human prion protein mutants by NMR

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Prion diseases are fatal neurodegenerative disorders which can be of sporadic, genetic and infectious origin. In genetic forms of prion diseases, misfolding of cellular prion protein, PrP^{C}, into its pathological form, PrP^{Sc}, is caused by mutations in the human prion protein gene. Understanding of the earliest stages of the conformational changes leading to spontaneous generation of prions in genetic forms of prion diseases may benefit from detailed structural characterization of various human (Hu) PrP variants. NMR spectroscopy is a powerful tool for providing information on three-dimensional (3D) structural features at the atomic level. Therefore, we undertook solution-state NMR studies of HuPrPs with pathological Q212P [1] and V210I [2] mutations linked to Gerstmann-Sträussler-Scheinker (GSS) syndrome and genetic Creutzfeldt–Jakob disease (CJD), respectively and of HuPrP carrying naturally occurring E219K polymorphism [3] considered to protect against sporadic CJD (sCJD).

Nearly complete backbone and side-chain assignments along with a high number of NOE distance restraints enabled high-resolution structure determination of HuPrP variants. None of the mutation affects the overall fold of HuPrP which highly resembles that of the WT protein consisting of unstructured N-terminal tail and a globular C-terminal domain. However, pathological Q212P and V210I mutations introduce several local structural differences in comparison to the WT HuPrP. Variations are mostly clustered at the α_2-α_3 interhelical interface and in the β_2-α_2 loop region leading to higher exposure of hydrophobic residues to solvent what may facilitate intermolecular interactions involved in spontaneous generation of prions. Notably, hydrophobic interactions in the β_2-α_2 loop region are not interrupted in HuPrP with protective E219K polymorphism. The NMR structures of HuPrP variants provide new insights into the possible key structural determinants underlying conversion of PrP^{C} to PrP^{Sc}.

In addition, we analyzed the effect of pH on the structural features of HuPrP(V210I) [4]. Comparison of NMR structures of HuPrP(V210I) at pH 5.5 and 7.2 revealed that the tertiary contacts are less perturbed at neutral pH conditions suggesting that spontaneous formation of prions may occur under acidic pH conditions in endosomal compartments.

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High-dimensional NMR experiment for sequential assignment in ^{13}C-labeled RNAs

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The determination of RNA structure by NMR spectroscopy is a challenging task, since it is often associated with severe spectral overlaps. The low proton density and less chemical-shift dispersion in RNAs makes the task even more difficult. The sequential resonance assignment in RNA is achieved using through-space NOE-type [1] or through-bond HCP-type experiments [2] and the efficacy of both types of experiments is severely affected by spectral overlaps, which further increases with the size of RNA.

Here we propose a novel through bond, non-uniformly sampled, H4'/C4' selective, four-dimensional HC(P)CH experiment which provides sequential connectivity via $H4'_{(i)}$-$C4'_{(i)}$-$P_{(i)}$-$C4'_{(i+1)}$-$H4'_{(i+1)}$ links. The experiment is designed with an emphasis on achieving higher resolution, reducing sensitivity losses and selective coherence transfer from ^{13}C4' to ^{31}P and from ^{31}P to ^{13}C4'. For obtaining reasonable resolution, non-uniform sampling is employed in indirect evolution of t_1 (^{1}H) and t_2/t_3 (^{13}Cs). To reduce sensitivity losses, multiple quantum coherences are preserved during evolution/transfer delays. Selective inversion pulses are used for selective coherence transfer and prevent C-C coupling evolutions. An interesting aspect of the experiment is the suppression of auto (out-and-back) peaks and enhancement of the cross (out-and-stay) peaks, which further facilitates the unambiguous resonance assignments in RNAs. The performance of the experiment was tested on a fully ^{13}C,^{15}N-labeled 34-nt hairpin RNA consisting of two A-RNA form stems, one adenine bulge, an asymmetric internal loop and a GAAA terminal loop [3].

The proposed experiment complements the set of two recently reported high dimensional experiments, 5D-APSY HCNCH [4] and 4D-NUS C(aro),C(ribo)-NOESY [5] for resonance assignment in RNAs.

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Probing the conformation of (1→2)-C-disaccharides by NMR

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The C-glycosides are compounds in which the interglycosidic oxygen atom is replaced with a methylene group to form glycoside analogs that are stable to enzyme or chemical hydrolysis. They belong to an important class of carbohydrate mimics that often exhibit a range of interesting biological properties [1–3]. Recently, we have developed a modular synthetic approach to a variety of (1→2)-C-glycosides (Figure 1) employing challenging sp^2-sp^3 Suzuki/Miyaura cross-coupling reaction [4].

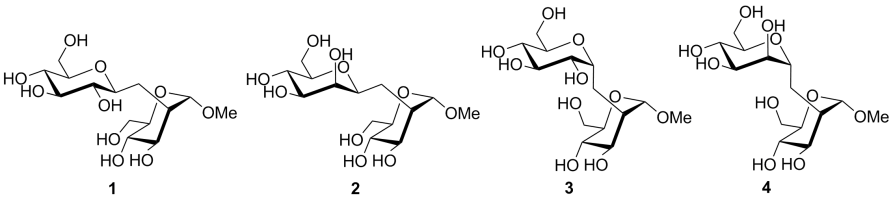

Figure 1: Structure of of studied C-glycosides.

The structure of the prepared compounds was studied by NMR. The configuration and conformation of individual saccharide units arise from an analysis of the vicinal coupling constants of ring protons. It was found that both D-mannopyranose and D-glucopyranose unit retains the same $^4C_1$ chair conformation in all final C-disaccharides. The overall conformation of C-glycosides was explored using NMR and molecular modeling [5]. Conformational space was explored by systematic variation of torsional angles φ and ψ, reflecting mutual rotation of saccharide units. The most stable conformers were optimized using DFT B3LYP/6-31G* method in water (polarizable continuum model). Probability distributions of individual conformers were obtained from calculated energies (3 kcal/mol energy cutoff) according to the Boltzman distribution. Optimized geometries of conformers provided also theoretical $^3J_{H,H}$ of the protons from methylene bridge encoding the C-disaccharide conformation. The weighted values of the $^3J_{H,H}$ according the Boltzman distribution were in agreement with observed ones.

Acknowledgement: This work was supported by the Czech Science Foundation (Grant No. 13-24880S and P207/12/P713).

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Sparsity in NMR and around

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Many scientific or technical problems are solved according to the following scheme:

1. Costly sampling of a signal
2. Finding a "sparse" representation of a signal
3. Signal compression.

NMR spectroscopy may serve as an example, where days-long measurements of millions of points of free induction decay signal are needed to find a few hundreds of spectral peaks, or costly sequencing of genome performed to find subtle changes caused by medical disorders. The following question arises: is costly data collection (point 1) really required, if it is compressed later on (point 3)? Rapidly developing field of numerical analysis known as "compressed sensing" provided an effective tool to solve these and other problems. The review of key concepts of the theory and its applications in a broad range of problems from chemistry, biology and technology will be presented during the talk.

Acknowledgements: For the financial support, the National Centre of Science (SONATA BIS 2 grant) and Foundation for Polish Science (TEAM programme) are kindly acknowledged.

Chemical exchange saturation transfer (CEST) MR imaging

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Methods of magnetic resonance imaging (MRI) have originally been designed to provide morphological images with sufficient contrast between normal and pathological tissues. Like in other NMR experiments, signal intensities in traditional MRI scans are dimensionless relative numbers and can be affected by various physical properties of free (mobile) water in tissues such as T_1 and T_2 relaxation times, apparent diffusion constants, or rates of magnetization transfer between free water and water bound to macromolecules.

Nowadays, researchers and clinicians become interested not only in morphology of tissues but also in their physical and biochemical properties, which can be assigned to specific pathological conditions. It means that image signal intensities should correspond to physical or chemical quantities (parameter maps). The easiest way of obtaining parameter map is to measure a series of images with varying particular pulse sequence parameter, e.g. an echo time for T_2 maps, an inversion time for the T_1 maps, a diffusion gradient for diffusion maps, etc.

The traditional way of measuring biochemical parameters (metabolite concentration maps) is spectroscopic imaging. However, MR signal intensity of important brain, muscle or liver metabolites is low, which results in long measurement times and low spatial resolution. Recently, an alternative way of measuring metabolic maps was suggested [1]. It is based on magnetization transfer between exchangeable protons of metabolites or biomacromolecules (–OH or –NH) and free tissue water. If a specific exchanging proton is selectively saturated or inverted, its non-equilibrium magnetization is transferred by chemical exchange to water and causes a decrease of water magnetization, which is then used for imaging. After a proper calibration, the decrease in water signal coming from individual voxels can be related to the concentration of the specific exchangeable protons. This CEST effect can be substantially enhanced by prolonged or repeated saturation so a measurable decrease in water signal can be observed even at low concentrations of exchangeable protons.

Changes in creatine concentration in exercising muscle were measured in a high resolution CEST MRI experiment by saturating its $-NH_2$ protons at 1.8 ppm downfield from water. The dynamic changes in the creatine concentration were in good agreement with the ^{31}P spectroscopic data [2]. The fast chemical exchange between –OH protons of glycosaminoglycans resonating at about +1.4 ppm and water enabled to perform a CEST experiment for determining concentrations of glycosaminoglycans in articular cartilage of knee [3]. Depletion of glycosaminoglycans in articular cartilage is considered an early marker of its degeneration.

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Can we do mouse brain histology *in vivo* using MRI?

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Animal models are widely used in biomedicine to investigate pathologies of the nervous system and to search for their effective treatments. Specifically, white matter (WM) degeneration is caused by many disorders including Multiple Sclerosis. Among many constituents of WM, myelin damage is the most pronounced and affects brain function. Myelin changes can be evaluated by magnetic resonance imaging (MRI) through T_2 and T_1 relaxation times [1–4]. The inversion recovery ultra short echo time pulse sequence (IR-UTE) [3] has been shown to be well suited for T_1 quantification measurements *in vivo*. However, imaging of the whole brain is challenging using these techniques due to long total acquisition time.

Recently, Bock et al. [4] suggested MP-RAGE pulse sequence for high resolution and time efficient imaging of mouse brain that yields high quality myelin maps including cortical white matter. Feasibility study of application of the segmented MP-RAGE pulse sequence and cryo-coil for contrasting WM/GM and quantification of T_1 in mouse brain *in vivo* (normal and with cuprizone induced demyelination), as compared to room temperature brain surface coil will be discussed.

Fig. 1 shows the comparison of selected horizontal slices obtained at two different inversion times (TI), illustrating switching of contrast between GM and WM, due to nulling of the signal. Calculated T_1 map exhibiting fine details in different regions of the brain is presented as well.

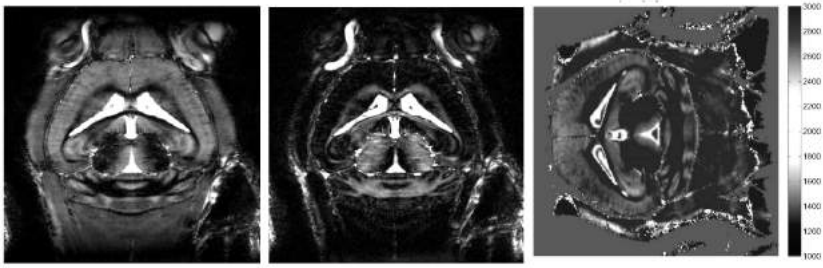

Figure 1: MP-RAGE images (TI = 900 ms and 1000 ms) and the T_1 map of the normal mouse brain obtained from cryo-coil.

According to our experience so far, the MP-RAGE sequence used with a cryo-coil at 9.4 T, thanks to a significantly higher SNR (2-3 times) than that achievable from a dedicated room temperature brain coil, allows one to obtain high resolution images of the whole mouse brain *in vivo*. WM/GM contrast is easily adjustable through the choice of appropriate inversion time, and experimental time is very attractive for *in vivo* experiments. The proposed MP-RAGE/cryo-coil setup is very promising for quantitative assessment of myelination in mouse models and potentially for *in vivo* mouse brain histology.

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Non-uniform sampling meets DOSY

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Multidimensional diffusion-ordered NMR spectroscopy is a useful tool for analysis of chemical mixtures. However, the method is limited by a long experimental time. In this study, we show how the limitation can be circumvented by an application of joint sparse sampling of both time and gradient domain. The signal processing exploits the sparsity-enforcing properties of ℓ_1-norm minimization. The approach was tested on 3D HSQC-DOSY spectra of two model mixtures. For both experiments the use of sparse sampling shortened the experimental time and improved the quality of the reconstructed spectra compared to the classical approach [1].

Acknowledgements: For the financial support, the National Centre of Science (HARMONIA grant) and Foundation for Polish Science (TEAM programme) are kindly acknowledged.

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Poster Presentations

T_2* relaxometry of thalamus in multiple sclerosis

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There is growing interest in the significane and impact of brain iron in multiple sclerosis. It has generally been shown that patients with MS often show T_2 hypointensity or other MRI changes suggestive of iron deposition in gray matter areas [1, 2]. A suitable parameter for monitoring tissue iron is T_2*, which is caused by a combination of spin-spin interaction and magnetic field inhomogenity [3]. We investigated twenty eight subjects with clinically defined MS (mean EDSS of 3.44, range 1–8) and fifteen age-matched healthy controls on 1.5 T scanner with multi-echo multi-slice GRE sequences with the aim to observe iron deposits in the thalamus.

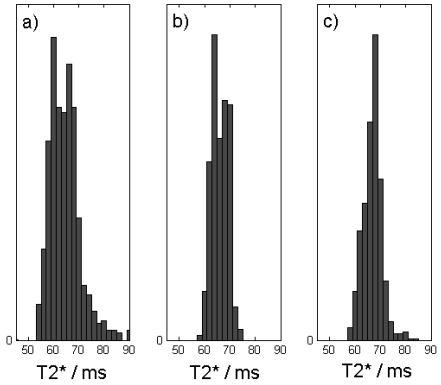

Figure 1: Distribution of T_2* values in left thalamus in age matched: multiple sclerosis female patients with a) EDSS = 3.5, b) EDSS = 1, and c) healthy volunteer.

We observed an increasing T_2* distribution width with increasing EDSS. Significant correlation of standard deviation ($p < 0.001$) and interquartile range (between 25th and 75th percentile) ($p < 0.001$) with EDSS was found. On the other hand, we did not observe any correlation of T_2* mean with EDSS. Our results indicate that instead of the commonly used averaging of T_2* values, it is more relevant to look at particular T_2* distributions as a whole.

This work was supported by project "Competence Center for research and development in the field of diagnostics and therapy of oncological diseases", ITMS: 26220220153, co-funded from EU sources and European Regional Development Fund.

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New point of view in utilising tags for structural analysis of complex mixtures

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Even the most powerful multidimensional (nD) NMR methodologies alone cannot solve structures of compounds contained in mixtures of thousands of small molecules. Some form of "spectroscopic separation" is therefore required. To achieve this, we have developed isotope-filtered nD NMR spectroscopy [1] that allows structural investigation of isotopically tagged molecules in complex, inseparable mixtures.

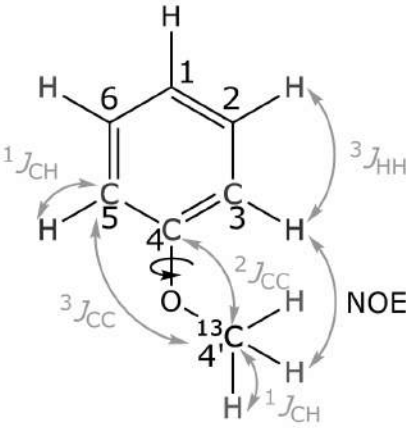

Figure 1: Polarisation transfer scheme

Incorporating isotopically labelled moieties within targeted functional groups of small organic molecules opens a unique possibility for structure characterisation of molecules in complex mixtures. Although isotopic tagging has been used in the analysis of mixtures in the past, we propose a new paradigm. Rather than focusing on the chemical shifts of the tags only, we use the tags for accessing chemical shifts and coupling constants of the parent molecules. We illustrate this approach by ^{13}C-methylation of hydroxyl and carboxyl groups in combination with purpose-designed nD NMR experiments.

Scheme on the right shows the interactions that can be used to transfer the polarisation in a ^{13}C-methylated phenol. These couplings were utilised in the design of nD NMR experiments that provide multiple correlated chemical shifts and coupling constants of aromatic rings nuclei. In these experiments, the signals from unlabelled molecules are eliminated, providing much needed simplification of NMR spectra of complex mixtures. The obtained information enabled the structures of derivatised compounds to be solved.

The developed methodology is aimed at the analysis of the aromatic moieties of humic substances (HS), the main organic component of soil and amongst the most complex mixtures on Earth. We have recently applied it to characterise major substitution patterns of phenolic moieties of fulvic acid isolated from a Scottish peaty soil [2].

Our approach is not limited to studies of HS, neither is the methylation as the way of introducing labels to report on the parent molecules. We are currently exploring several different approaches based on the principles outlined here.

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Binding abilities of new chiral reagents for separation of helicenes

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For the separation of unsubstituted racemic helicenes, a series of chiral reagents was designed and synthesised. The main idea was the synthesis of a chiral receptor, which would be capable of the formation of charge transfer complexes with unsubstituted helicene [1]. To bind an electron-rich helicene through the π-π interaction, some electron withdrawing groups were attached to the aromatics contained in the receptor [2]. For the subsequent industrial application, the synthesis shouldn't be very expensive. Therefore, some non-expensive or naturally chiral compounds were employed for chirality introduction into the receptor.

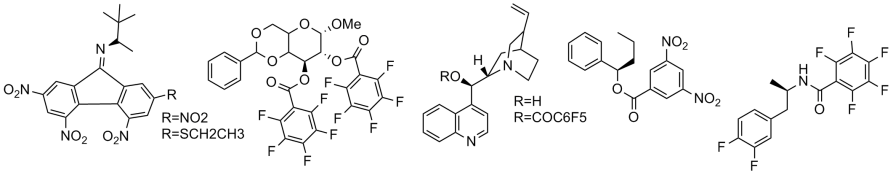

Figure 1: Structures of the receptors

For the comparison of binding properties of the prepared receptors, complexation constants3 towards pure [6]helicene as well as some derivatives of [6]helicene and [7]helicene were evaluated [3]. Based on preliminary ^{1}H NMR and ^{19}F NMR titration measurements, the receptors appeared to be capable of complex formation with helicenes and, also, of some discrimination against the individual derivatives.

Acknowledgments: for the financial support, the Technology Agency of the Czech Republic (TA04010082) is gratefully acknowledged.

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^{1}H NMR, CD and UV study of duplex-quadruplex structural hybrid

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Guanine-rich nucleic acid sequences fold into inter- and intramolecular structures known as G-quadruplexes. The core of G-quadruplex is composed of at least two stacking G-tetrad stabilized by cations (central metal ions). Quadruplexes play a fundamental role in gene expression and are implicated in important biological processes.

The main research objective of our work is the design of duplex-quadruplex structural hybrid. Using oligonucleotides with a dual functionality we are planning to develop a novel strategy of gene silencing, schematically presented in Fig. 1. These oligonucleotides will consist of two functionally independent domains: antisense domain which will bind to the target mRNA at its proper binding site and the second domain with two or three units of contiguous guanine runs designed to assemble into quadruplex by incorporation of a guanine rich region of the target mRNAs. We expect that quadruplex motif will contribute to the increased selectivity and stability of complex between antisense oligonucleotide and RNA target site [1, 2].

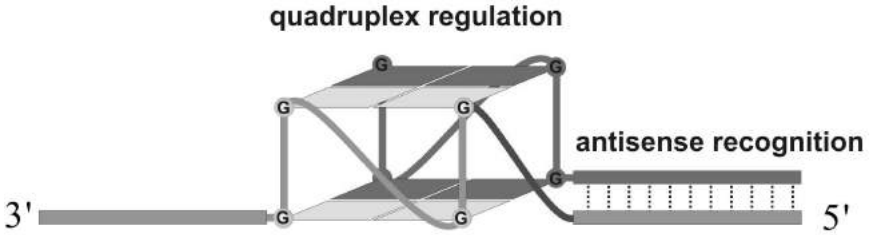

Figure 1: Schematic representation of duplex-quadruplex structural hybrid

Oligonucleotides will be chemically modified in order to improve the thermodynamic stability of the duplex motif and to promote the formation of quadruplex motif.

Our preliminary results obtained for antisense oligonucleotide where duplex part was modified with 2'-OMe residue and abasic linker was introduced into guanosine rich region will be presented.

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Analysis of complex reacting mixtures by time-resolved 2D NMR

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Nuclear magnetic resonance (NMR) spectroscopy is a versatile tool for chemical analysis. Besides the most straightforward application to study a stable sample containing a single compound, NMR has been also used for the analysis of mixtures. In particular, the analyzed mixtures can undergo changes caused by chemical reactions. The multi-dimensional NMR techniques are especially effective in a case of samples containing many components. Unfortunately, they are usually too lengthy to be applied in time-resolved experiments performed to study mentioned changes in a series of spectral "snapshots". Recently, time-resolved non-uniform sampling (NUS) has been proposed as a straightforward solution to the problem. In this paper we discuss the features of time-resolved NUS and give practical recommendations regarding the temporal resolution and use of the time pseudo-dimension to resolve the components. The theoretical considerations are exemplified by the application in challenging cases of fermenting samples of wheat flour and milk [1–4].

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High-dimensional ^{13}C-detected experiments for assignment of intrinsically disordered proteins

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High-dimensional ^{13}C-detected NMR experiments have been proven useful in protein studies [1]. In particular, the research on intrinsically disordered proteins (IDPs) gained much from the application of various techniques of this kind [1]. Due to high incidence of proline residues in IDPs the proton detected experiments are often infeasible. High flexibility of an IDP chain and high incidence of sequential repeats cause low peak dispersion and high dimensional experiments can alleviate this problem.

Here we present three new ^{13}C-detected experiments: 4D (HACA)CON(COCA)NCO, 5D HabCabCO(CA)NCO and HNCO(CA)NCO, which combined provide establishment of sequential links, recognition of glycine residues by peak signs and β chemical shifts (for amino acid recognition), thereby enabling assignment process. The pulse sequences take advantage of non-uniform sampling to maximize the achievable resolution and minimize the experimental time [2]. The proposed set of experiments was successfully applied to the sample of a resolution-demanding IDP: α-synuclein.

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Interaction of neuronal intrinsically disordered proteins with multiple binding partners studied by NMR

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Intrinsically disordered proteins (IDPs) have attracted a lot of attention in recent years, in particular because of the discovery of their importance in eukaryotic life and their central role in protein interaction networks. In contrast to their stably folded counterparts, IDPs lack stably folded tertiary structures. Indeed their intrinsic flexibility is of significant importance on their biological functionality. The sampling of a vast and heterogeneous conformational space endows them with enormous potential to interact with and control multiple binding partners at once. Yet, the way of interaction with their partners remains elusive and is a very active field of research.

We investigate IDP interactions with multiple and different molecules and their consequences on a cellular level. We focus on two proteins involved in neuronal plasticity, the Growth Associated Protein 43 (GAP-43) and the Brain Acid Soluble Protein 1 (BASP1) [1, 2]. These proteins are functionally related as they are both found in neurons where they sequester phosphatidylinositol-4,5-bisphosphate (PIP2) in lipid rafts. They undergo long-chain acylation, are substrates of Protein Kinase C (PKC), interact with calmodulin (although by very distinct modes) and bind to membranes. Surprisingly, although they share so similar functionalities, GAP-43 and BASP1 share no significant sequence identity. Therefore these two proteins are models of choice in order to understand how different (but functionally related) IDPs exploit their various conformational ensembles for accommodation of their respective partners. Using changes in chemical shift and relaxation rates combined with PRE data and other biophysical methods, we show the binding of the two IDPs to membrane systems, cognate protein binding partners as well as the impact of post-translational modifications (long-chain acylation and phosphorylation) on the structural dynamics of IDPs.

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NMR detection of the tautomeric equilibria for the substituted β-diketones

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β-Diketones are known to exist as two fast interchanging enolic tautomers (stabilized by intramolecular hydrogen bonding) through slow keto-enol tautomerism. The NMR spectra of the cis-enolic tautomer are the weighted average of the two forms. Therefore, both symmetric and non-symmetric *cis*-enolic forms give only one resonance for each type of nuclei, which makes it difficult to distinguish between these two forms.

To study the enol-enol tautomerism of the cis enol form of nonsymetric 1-phenyl-3-(3,5-dimethoxyphenyl)-propane-1,3-dione (compound **1**) and the ortho-dibromo derivative, the deuterium isotope effects on ^{1}H and ^{13}C chemical shifts of these compounds were measured. It is known that isotope effects on chemical shifts may occur in different ways: as the intrinsic effect, which is more common, or as a perturbation of equilibrium [1].

During deuteriation of compound **1**, the methine proton and hydroxyl proton were exchanged to get monodeutero products. From the variable temperature ^{1}H NMR spectra of compound **1** we observed that the coalescence temperature was 20℃. Additional information of the equilibrium was obtained from ^{13}C NMR spectra at low temperature. Eight signals in carbonyl carbon region were detected (Figure 1), which belonged to the enolic forms of two mono-deutero products. All these NMR data of equilibrium deuterium isotope effects indicated that the intramolecular hydrogen bond was in the double-well potential type with one minimum of lower energy than the other.

The introduction of a steric group (bromine atom) at the ortho positions of methoxy substituted phenyl ring changed the potential energy curve of the molecule, which was caused by the change of the electron density in the entire molecule. Contrary to compound **1**, parent dibromo derivative exists in only one localized cis-enolic tautomer exhibits intrinsic secondary deuterium isotope effect on ^{13}C chemical shifts. This is a clear indication of two completely different types of intramolecular hydrogen bond potentials present in β-diketones.

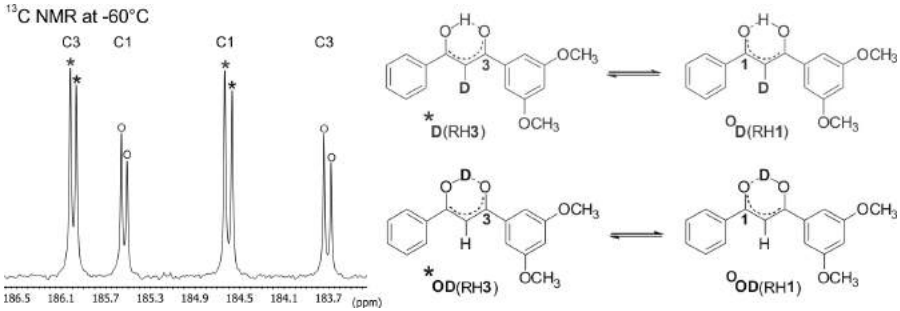

Figure 1: Carbonyl region of ^{13}C NMR spectrum of the monodeuterated products of **1**

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Study of micellization of surfactants by DOSY NMR

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Diffusion-ordered spectroscopy (DOSY) NMR experiments represent an attractive non-invasive method for the analysis of mixtures and the study of diffusion properties of molecules in solution. In DOSY NMR, spectral separation of mixture components is based on their different diffusion properties and hence on differences in their molecular size, radius, shape and/or intermolecular interactions. DOSY NMR techniques provide multi-dimensional maps in which one axis corresponds to the diffusion coefficient and the others to the chemical shift dimensions [1, 2]. Diffusion separation, as observed in pure solvent solutions, is, however, frequently not sufficient. One of the remedies is a modification of the solvent matrix by the addition of an appropriate inert agent, which can specifically interact with the solute molecules and in this way alter their diffusion properties. There are various classes of molecules exploited for this purpose. Among them, an important role belongs to surfactants.

Surfactants have an amphiphilic type of molecule containing hydrophilic and hydrophobic moiety. They self-associate in aqueous solutions to form aggregates of various types, shapes and sizes. At a certain concentration, called critical micellar concentration (cmc), micelle formation occurs. The size of surfactant molecules and thus their self-diffusion behavior change upon micellization [3]. The process of micellization can be monitored by the observation of concentration dependent changes of self-diffusion coefficients.

In this work we have explored the micellization process of some surfactants, with the aim of their exploitation for DOSY NMR spectroscopy.

Acknowledgement: The authors wish to thank VEGA 1/0770/15 for support.

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Processing of multidimensional data using multiple fixing SMFT method

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The sparse multidimensional Fourier transform processing algorithm (SMFT) [1] allows one to obtain a set of two-dimensional cross-sections from a non-uniformly sampled multidimensional experiment. It is possible by fixing frequencies of two (in case of 4D experiment) or three (in case of 5D experiment) types of nuclei. Fixed frequencies are collected from the spectrum (the "basis spectrum") that contains at least part of the frequencies measured in the high-dimensional experiment.

Here we present how fixing different frequencies from the basis spectrum, 4D (HACA)CON(COCA)NCO, enabled us to easily access information from multidimensional experiments: 4D (HACA)CON(COCA)NCO, 5D HNCO(CA)NCO and 5D HabCabCO(CA)NCO. This information was then used to find the sequential connectivities and assign peaks.

In addition, we compared the usefulness of various subsets of data obtained by multiple fixing of 4D (HACA)CON(COCA)NCO, 5D HNCO(CA)NCO and 5D HabCabCO(CA)NCO experiments in automatic chemical shifts assignment for alfa-synuclein, a resolution-demanding, intrinsically disordered protein 140 amino acids long, using the TSAR program [2].

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Spiro cycles from acridin-9-ylmethylamine: a NMR study

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A novel type of spontaneous cyclization of 1-alkyl/aryl-3-(acridin-9-ylmethyl)thioureas obtained from the title amine and alkyl/aryl isothiocyanates led to 1-alkyl/aryl-spiro[dihydroacridine-9'(10'H),5-imidazolidine]-2-thiones further transformed with mesitylnitrile oxide to spiro[dihydroacridine-9'(10'H),5-imidazolidine]-2-ones that partly reopened to urea isomers. Forty target compounds in three series were prepared to study their structure, mechanism of formation, and substituent effects by ^{1}H, ^{13}C, and ^{15}N NMR spectroscopy. Whilst all thioureas except bis-acridine ones cyclized at the acridine C-9 carbon via R^2—N nitrogen, bis-acridine precursors (R^2 = 9-Acr) cyclized via methylene carbon to give inverted spiro cycles. Transfer of substituent effects of para substituents on R^2 to the spiro ring was different between the thione and one derivatives, as it was demonstrated by induced shifts of the corresponding signals in ^{13}C and ^{15}N NMR spectra.

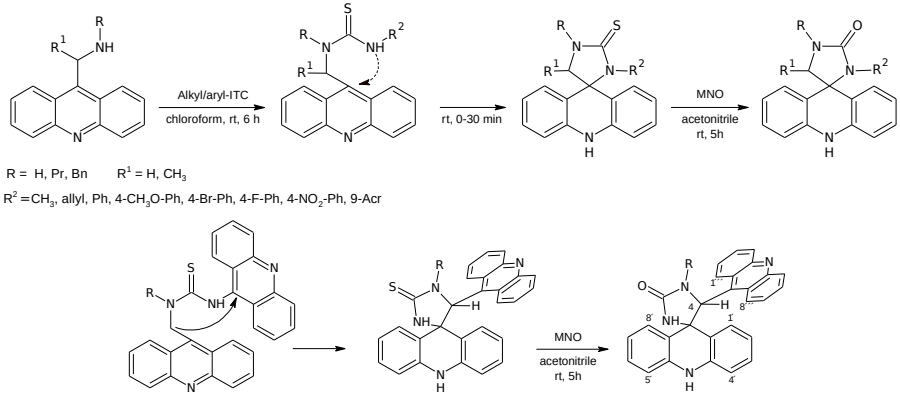

Figure 1: Reaction scheme

Acknowledgements: Grant VEGA 1/0672/11. The Slovak State Programme for Support of Research Infrastructure: NMR.

NMR characterisation of 1,5-bis(salicylidene)carbohydrazide in solution and solid state

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Carbohydrazide derived Schiff bases are higher urea homologues with interesting biological properties, which together with their reactivity, led to a number of laboratory and industrial applications. These compounds have been widely used as selective analytical reagents for ion binding [1], as precursors in the syntheses of nitrogen containing heterocycles [2] and as multitopic ligands for the targeted construction of coordination systems with biological activity [3]. They are usually prepared by the condensation reaction between carbohydrazide and appropriate carbonyl compound.

The structure of 1,5-bis(salicylidene)carbohydrazide **1** has been characterised by NMR spectroscopy in solution and solid state. As shown in Figure 1, this molecule might exist in several tautomeric forms and has a few reactive groups able to participate in both intra- and intermolecular hydrogen bonds. It has been demonstrated that the tautomer **1a** is the dominant form in solution and in all solid phases. Significant deshielding and line-broadening of the signals corresponding to NH and OH protons have clearly indicated that both groups formed hydrogen bonds. Furthermore, the analysis of solid-state NMR spectra allowed the identification of five crystallographic forms of **1**: three polymorphs (**I**, **II** and **III**) and two solvates (**IV** and **V**) [4]. The obtained results can further be exploited for better understanding the relationship between the structure, physico-chemical properties and bioactivity of carbohydrazide derivatives.

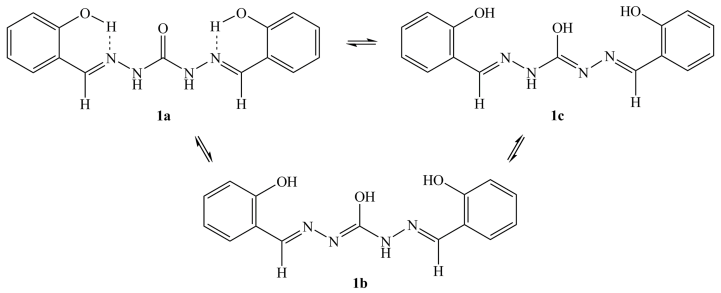

Figure 1: Tautomeric forms of **1**

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NMR spectroscopic studies of de/protonation mechanisms in thiosemicarbazide and azobenzene based anion chemosensors

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Four thiosemicarbazide anion chemosensors containing three N—H groups, substituted with phenyl and/or 4-nitrophenyl units, were synthesized and studied for their anion binding abilities with OH^-, F^-, CH_3COO^-, $H_2PO_4^-$ and Cl^- anions. Noteworthy changes were observed in the UV-visible absorption spectra upon addition of all anions, except for chloride, accompanied by dramatic color changes visible to the naked eye. These changes were determined to be due to the deprotonation of the central N—H proton and not due to hydrogen bonding, based on ^1H-^{15}N NMR titration studies with acetate in DMSO-d_6/0.5% water. Deprotonation was confirmed by the disappearance of NMR signal for the central thiourea H_2 proton and the formation of acetic acid. In addition, long-range ^1H-^{15}N NMR experiments allowed unambiguous assignment of ^{15}N chemical shifts, where aromatic N_1 atom was strongly deshielded from δ 106 to 158 ppm. Therefore, the thiosemicarbazide anion chemosensors operate by a deprotonation mechanism of the central N—H proton rather than by hydrogen bonding as is often reported [1].

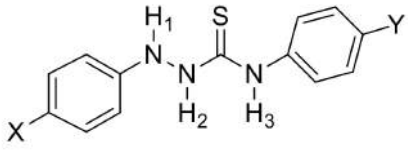

Figure 1

Three azobenzene based pH indicators with amino[bis(ethanesulfonate)] substituents were synthesized and preliminary studied by UV-visible absorption spectroscopy in aqueous solution. The azobenzene indicators exhibit brilliant and distinct color changes with transitions between pH 1 and 4. The UV-visible spectra suggest that the protonation site is a tautomeric equilibrium between protonation at the azo nitrogen and protonation at the amino nitrogen. NMR spectroscopic studies, which include ^1H and ^{15}N NMR titrations, are in progress to provide unambiguous proof of the protonation site.

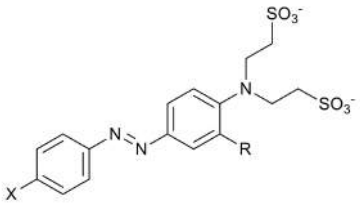

Figure 2

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Faster and cleaner real time pure shift NMR experiments

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^{1}H homonuclear broadband decoupling has seen a lot of attention in the recent years. Pure shift proton NMR experiments, obtained by homonuclear broadband decoupling, significantly enhance the resolution of scalar coupled signals. The rediscovery of older methods towards pure shift spectra, like slice-selective decoupling [1] and the BIRD experiments [2] and their further development [3–6] have established them as useful tools in modern liquid NMR specroscopy. However, improved resolution is traded against significantly reduced sensitivity and introduction of artifacts.

Here, improvements to the real time slice-selective decoupling method are presented. The experiment now includes the suppression of artifacts originating from scalar coupling evolution during the acquisition of data chunks. The frequencies of these artifacts depends on the length of the recorded data chunks, therefore suppression is achieved by cycling the data chunk lengths over a defined number of scans. This leads to the collapse of the sharp artifact signals to very broad signals with significantly lower intensity.

The second improvement is the use of frequency shifted pulses. By concertedly shifting the offsets of the employed selective pulses, the need to wait for relaxation of slowly relaxing protons is reduced. This applies to molecules as well as to compound mixtures featuring protons with a broad range of T_1 relaxation times.

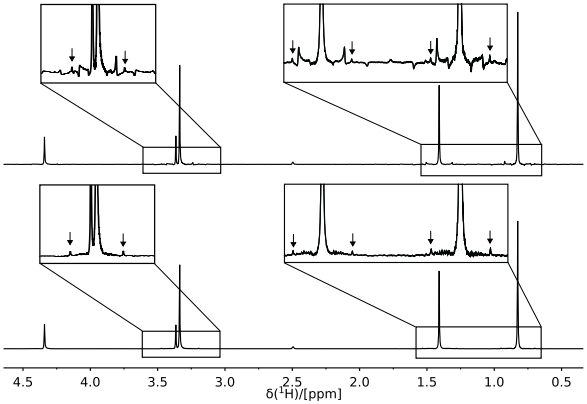

Figure 1: Real time slice-selectively decoupled ^{1}H spectra of 10% *n*-propanol in DMSO-d_6 with (bottom) and without (top) the use of artifact suppression. The arrows in the enlarged areas indicate the ^{13}C satellites.

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The solution structure of the MANEC-type domain from hepatocyte growth factor inhibitor 1 reveals an unexpected PAN/apple domain-type fold

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A decade ago, the **m**otif **a**t **N**-terminus with **e**ight **c**ysteines, or in short MANEC, was defined as a new protein domain family. These domains were found exclusively in the N-terminus of > 400 multi-domain membrane proteins from multicellular animals. Despite the large number of MANEC-containing proteins, only one has been characterized: hepatocyte growth factor activator inhibitor-1 (HAI-1). HAI-1 is an essential protein shown to regulate the activity of matriptase, hepsin and hepatocyte growth factor activator, all serine proteases with crucial roles in epithelial development, cell growth and homeostasis. Misregulation of these systems has been implicated in severe pathological conditions such as skin diseases and cancer. Detailed functional understanding of HAI-1 and other MANEC-containing proteins is hampered by a lack of any structural information on MANEC.

Here we present an NMR solution structure and biophysical characterization of the MANEC domain from HAI-1, as the first structure of a representative MANEC domain. Although no homologies were predicted based on sequence, the MANEC structure revealed it as a new subclass of the PAN/apple domain family. Intriguingly, in silico protein folding resulted in successful structure-based homology prediction, where sequence-based approaches fail. The MANEC structure represents a much needed tool for elucidation of function of MANEC-containing proteins as indicated by the homology to the PAN/apple domains as mediators of protein-protein and protein-glycan interactions.

Authors wish to thank the Foundation for Polish Science for the support with TEAM programme "Towards new applications of NMR spectroscopy in chemical and biomolecular structural studies" (M.N., W.K.), NMR measurements were carried out at the Biological and Chemical Research Centre, University of Warsaw, established within the project co-financed by European Union from the European Regional Development Fund under the Operational Programme Innovative Economy, 2007–2013.

Magnetic resonance access to transiently formed protein complex

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Biomolecule interactions are important to understanding of biological phenomena since non-covalent reversible binding between proteins and other biomolecules leads to the formation of complex regulatory molecular systems. These interactions, however, can exist over a wide range of dissociation constants and time scales, illustrating interactions in tight complexes, as well as weak transiently formed protein complexes.

CspA is one of the cold-shock proteins naturally occurring in *E. coli.* The CspA protein has been shown to bind to the 5' end UTR of its own mRNA, called the cold box (CB), and destabilize its secondary structure acting as a chaperone. The interaction of the CspA with RNA is a transient and sparsely populated binding event that can be studied by the means of CPMG relaxation dispersion NMR experiments. Chemical shift changes upon binding of the cold box and its complementary strand anti cold box RNA were observed finding out that mostly surface-exposed hydrophobic residues are involved in the binding event, suggesting that the protein-RNA interaction is facilitated through hydrophobic interactions.

Sparsity-constrained NUS reconstruction in NMR: possible pitfalls

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Non-uniform sampling (NUS) has been widely applied to accelerate multidimensional NMR experiments. A group of signal processing methods reffered to as compressed sensing (CS) has been proved especially effective in the reconstruction of NMR spectra from NUS data. All the CS methods are based on the assumption that the recovered spectrum is sparse, i.e. that it contains a relatively small number of significant components. The search for the sparsest spectrum x that fits to the measured experimental time-domain data y requires to set the balance between sparseness of x (quantitavely expressed by $f(x)$) and its accordance with the undersampled FID signal y. This is realised by minimising the function

$$\min_{x} \left(\|\mathcal{F}x - y\| + \lambda f(x) \right),$$

where $\mathcal{F}$ stands for inverse Fourier transform, and λ sets the balance between the sparsity of the spectrum and the agreement with the measurement.

In this study, we discuss the effects of missetting the reconstruction parameters (e.g. λ, stopping citeria) in various CS methods: orthogonal matching pursuit [1], known in NMR under the name CLEAN [2], Lorentzian peak matching pursuit [3], Iterative Soft Thresholding [4, 5], iteratively reweighted least squares [5] and Low-Rank reconstruction [6].

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G-quadruplexes: formation of long-lived intermediates

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G-quadruplexes are higher order secondary structures formed by guanine-rich DNA sequences that can be found in biologically significant regions of the genome such as telomeres, immunoglobulin switch regions and promoter regions of eukaryotic cells. They have also been associated with human diseases, as therapeutic targets in drug design and in applications as nanomolecular devices. The presence of cations such as K^+ or Na^+ seems to be essential for the formation of G-quadruplexes, due to their role in reducing repulsions amongst guanine carbonyl oxygen atoms within G-quartets and additionally enhancing base-stacking interactions.

With the use of solution-state NMR spectroscopy and other experimental techniques (PAGE, TDS, UV, CD, DSC) we have studied the behaviour of guanine-rich sequences and explored their features in an environment almost completely free of G-quadruplex promoting cations. Experimental data has shown the formation of a new structure, which can be considered as an intermediate on the way to folding into G-quadruplexes. It is interesting to note that the guanine bases are not held together by Hoogsteen hydrogen bonds like in G-quartets but rather by alternative base pairing.

Our study, where G-rich intermediates were characterized in detail and their kinetic roles determined, provides an important step in elucidating general principles by which G-quadruplexes adopt their native folds [1]. G-rich DNA sequences can in the presence of certain cations fold very rapidly and not by chance in to a large number of structurally diverse G-quadruplex structures with mechanisms of varying complexity through the population of different intermediates, which are generally unstable and hard to detect.

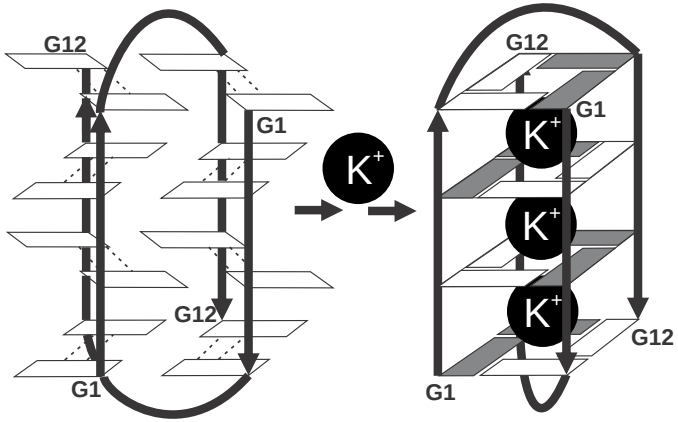

Figure 1: Oxy-1.5 G-quadruplex folding step involving i-Oxy-1.5 intermediate. Guanine residues at the 5' and 3' ends in both species are marked.

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NMR detection of tautomeric equilibria for substituted β-diketones

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Cytoskeletal actin-binding protein α-actinin 1 is a member of the spectrin superfamily with the role to cross-link the actin filaments. It is a calcium sensitive non-muscle cytoskeletal isoform of α-actinin. The protein is functional as an antiparallel dimer, which is composed of an N-terminal actin-binding domain, connected through a flexible neck peptide to four spectrin repeats forming the central rod that is followed by a C-terminal calmodulin-like (CaM) domain [1]. The CaM-like domain of α-actinin 1 involves four EF-hand motifs that could bind up to four calcium (Ca^{2+}) ions. It is believed that calcium binding triggers a major conformational change of the CaM-like domain of α-actinin 1 from a closed into an open state. In this way, α-actinin 1 is able to interact with different target partners depending on its calcium binding state.

Triple resonance (^{1}H, ^{13}C and ^{15}N) NMR experiments on sample containing 1 mM uniformly labelled CaM-like domain of α-actinin 1 were performed on an 800 MHz NMR spectrometer.

CaM-like domain of the α-actinin 1 is composed of two lobes, one in N- and other in C-terminal. N-terminal lobe consists of two EF-hand motives connected with linker. EF-loops, which connect internal and external helices of each EF-hand motives are close in space and connected by a short β-sheet. C-terminal lobe consists of four helices, three of them are shorter in comparison with helices in N-terminal lobe and do not form EF-hand motives. In apo form, no single preferred orientation of N- and C-terminal lobes was observed. CaM-like domain is stabilized after titration with Ca^{2+} ions. We suggested that calcium binding triggered the stabilization of structure trough limitation of the rotation of the N- and C-terminal lobes around the linker between them.

Titration of protein sample with Ca^{2+} ions followed with ^{15}N-HSQC spectra proposed one binding site for Ca^{2+} ions in the first EF-hand motif in the N-terminal lobe of CaM-like domain. After comparison of the apo and holo structures, for individual lobes no major structural rearrangements were observed as consequence the calcium binding. In the holo form internal and external helices of first and second EF hand motives in N-terminal lobe are slightly opened compared to the apo form, but not as much as is known for EF-hand proteins with similar function, such as calmodulin [2]. The most significant changes between both forms were observed in EF-loops of both EF-hand motives and in linker connecting them in N-terminal lobe. Our NMR studies were complemented by isothermal titration calorimetry, mass spectroscopy and SAXS.

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Residual dipolar coupling assisted NMR and DFT analysis of an exotic product of Povarov reaction

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During a study of the mechanism and the synthetic possibilities of the Povarov reaction [1, 2], a cyclization of (2'*R*,3*S*)-1'-benzyl-2'-(prop-2-en-1-yl)spiro[indole-3,3'-pyrrolidine]-5'-one (**1**) was performed. The reaction was attempted with various Lewis and Brønsted acids as catalysts, with ethylaluminium dichloride giving the best yield.

Due to the improbable exotic alkaloid-type structure of the resulting molecule **2**, a detailed structural analysis was called for. For this reason, a set of 2D NMR spectra including INADEQUATE was measured in order to prove the atom linkages. The bond configurations of **2** were predicted from the synthesis mechanism and confirmed unambiguously using NOESY, DFT geometry calculations, and ultimately RDC assisted NMR analysis in stretched crosslinked polystyrene gels [3]. The reversibility of the cyclization, which is typical for Diels-Alder type reactions, was observed, thus complicating the RDC analysis because of long swelling times of the polymer gels.

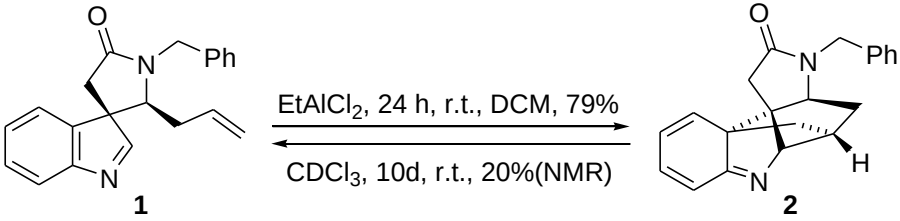

Figure 1: Synthesis scheme of **2**

Acknowledgement: The authors wish to thank VEGA 1/0770/15 for support.

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Experimental determination of structural parameters in selected polycyclic aromatic compounds

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The unusual optical and electronic properties of polycyclic aromatic compounds have origin namely in the conjugated π-π aromatic system [1]. These unique systems are mostly studied by theoretical approaches. Here we would like to present interconnection between the experimentally accessible data and the structural parameters in helicene series of aromatic compounds. ^{1}H and ^{13}C NMR chemical shifts and their temperature dependence can be correlated with the interplanar distance of peripheral helicene rings and reflect the dynamic behaviour of the molecule.

The indirect spin-spin ^{13}C-^{13}C coupling constants represent unique probe to the electron distribution between involved atoms and later reflect the carbon-caron bonding environment [2]. The experimental values of ^{1}J can be correlated to the carbon-carbon bond lenghts and angles, that are accessible by single crystal X-ray crystallography. The experimental values of ^{3}J(C—C) correlates reasonably well with the published theoretical study of helicene aromaticity [3]. The theoretical prediction of NMR parameters was done using DFT calculation (B3PW91/6-311++G(d,p)) which provides an excellent agreement with the experimental values.

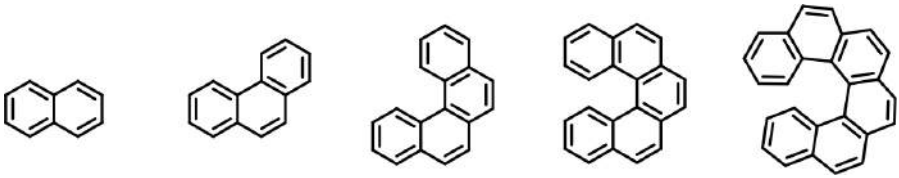

Figure 1: Helicene series of aromatic compounds

Acknowledgements: For the financial support, the Czech Science Foundation (Project No. 15-12719S) is gratefully acknowledged.

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Electron paramagnetic resonance study on co-phthalocyanine ionic derivative spontaneously adsorbed on highly ordered pyrolitic graphite (HOPG)

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Electron paramagnetic resonance (EPR) belongs to the family of magnetic resonancies and focuses on materials and compounds with unpaired electron. EPR is very well known and established among other spectroscopic techniques, just as old as a nuclear magnetic resonance (NMR). It is also suitable for surface analysis, bacause it can provide direct qualitative as well as quantitative evidence of the unpaired electron (spin) distribution on a surface, which cannot be acquired by common surface methods (e.g. XPS or SPM). Electron paramagnetic resonance is frequently applied in the field of transition metal oxide surfaces [1, 2]. However, it has been rarely applied in the field of adsorption onto conducting/semiconducting substrates.

We found that the adsorption of a 4-fold positively charged ionic phthalocyanine: [N,N',N",N"'-tetramethyltetra-3,4-pyridinoporphyrazino-Co(II)], directly from its corresponding solution, can be followed by in situ EPR spectroscopy in the EPR cell. Our combined spectroscopic and quantum chemical computational (by density functional theory, DFT) analysis show that the above-mentioned phthalocyanine differs in geometry, depending on either being in powder or adsorbed on HOPG. Additionally, it was found that the spin state of HOPG conducting electrons does not change upon adsorption [3]. Finally, by such an spontaneous adsorption process from corresponding phthalocyanine solution, one can get homogeneous, magnetically and redox active phthalocyanine molecular film, which may find an applicaation in molecular electronis (sensorics) or as a magnetic resonance imaging (MRI) contrast agent.

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Kaempferol glycosides from the leaves of *Lotus japonicus*

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The genus *Lotus* includes more than 180 species that are found worldwide except in very cold regions [1]. Different species of *Lotus* are currently used to improve pastures and hay quality where other forage legume species are not suitable [2].

Four known flavonol glycosides, i.e., kaempferol 3,7-O-α-L-dirhamnopyranoside (**1**), kaempferol 3-O-β-D-glucopyranoside-7-O-α-L-rhamnopyranoside (**2**) [3], kaempferol 3-O-β-D-glucopyranosyl-(1⟶2)-β-D-glucopyranoside-7-O-α-L-rhamnopyranoside (**3**), and kaempferol 3-O-β-D-glucopyranosyl-(1⟶2)-β-D-galactopyranoside-7-O-α-L-rhamnopyranoside (**4**) were isolated from the leaves of *Lotus japonicus* Gifu B-129 (Figure 1), and the structures of flavonol glycosides were deduced from high field 1D and 2D NMR spectra. Compounds **3** and **4** were isolated from *Lotus japonicus* for the first time.

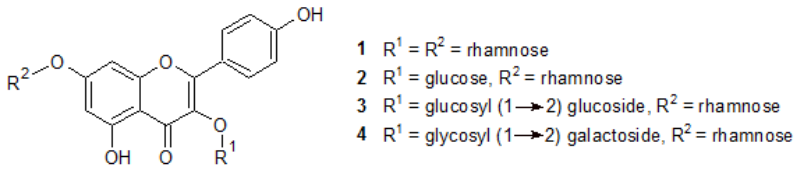

Figure 1: Structure of kaempferol derivatives

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Solid state ^{13}C NMR studies of modified poly(3-hydroxybutyrate)

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The substitution of conventional polymeric materials by biodegradable materials is one of the most attractive recent trends in material science. PHB (polyhydroxybutyrate) belongs to biologically synthesized polymers. It is non-toxic, biocompatible, biodegradable, and thermoplastic material. PHB is semicrystalline [1] polymer and its mechanical properties are similar to those of isotactic polypropylene, except for elongation at break leading to low toughness. Moreover, under ambient conditions its properties change significantly due to physical aging [2]. On the other hand, appropriate modifications of PHB provide material with unique mechanical properties.

Properties of polymeric materials are determined by their structure and interactions at molecular as well as supramolecular grade, and molecular dynamics which is closely related to the structure. This work studies the influence of thermal treatment and plasticization on the structure and molecular dynamics of PHB by means of nuclear magnetic resonance (NMR) spectroscopy, which is a unique tool for the study of the structure and molecular dynamics of polymeric materials. For this purpose, the single pulse MAS ^{13}C NMR spectra at ambient temperature and at 370 K, and spin-lattice relaxation times $T_1(^{13}C)$ were measured for three PHB samples: virgin (PHB-v), quenched (PHB-Q) and plasticized with triacetine (PHB-QT).

The solid-state ^{13}C NMR spectra measured for all studied samples at both temperatures display carbonyl (CO), methine (CH), methylene (CH_2) and methyl (CH_3) resonances with narrow lines arising from crystalline domains superimposed on broad lines from amorphous domains. The spin-lattice relaxation time $T_1(^{13}C)$ determined from measurements using the Torchia technique [3] showed bi-exponential decay for each resonance and besides that it was found out, that the used delay time of 120 s was long enough only for already relaxed CH_3 group. Both used measurement techniques imply that all studied PHB samples have semi-crystalline nature. The changes of the structure and molecular motion in PHB were further analyzed by deconvolution of the methyl carbon resonance.

Thermal treatment and plasticization slightly influence the crystallinity of PHB at ambient temperature. Triacetine in PHB caused significant narrowing of the linewidth of the resonance line associated with amorphous domains at ambient temperature, which indicates the narrowing of the conformation distribution. The same effect was observed for all samples at the temperature of 370 K, but this time it could be related to the change of mobility in amorphous domains. The most significant change in crystallinity forced by temperature was found in PHB and PHB-Q.

Acknowledgement: This research has been supported by the Scientific Grant Agency (VEGA) of the Ministry of Education of the Slovak Republic and the Slovak Academy of Sciences through the project No. 1/0492/13.

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Application of alignment media in structural analysis of calix[4]arene derivatives

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The aim of this work is the application of residual dipolar coupling constants (RDCs) [1, 2] measurement method in conformational behavior study of new inherently chiral calix[4]arene derivatives. RDC is an anisotropic direct through space dipole-dipole interaction, which is not observable in isotropic solution due to time averaging caused by fast molecular reorientations. This interaction could be reintroduced by partial alignment of the compound in external magnetic field and is manifested as the contribution to a scalar coupling constant. The magnitude of this interaction is dependent on the distance of coupled nuclei and includes information about their spatial arrangement.

The study was started with the testing of the series of recently published liquid crystalline alignment media based on polyglutamates and polyacetylenes on the model dipropoxycalix[4]arene with respect to the ease of the preparation and the degree of the introduced alignment. Suitable alignment media were used to determine or specify the spatial structure of three types of new inherently chiral calix[4]arene derivatives, whose structures could not be determined by standard NMR methods (NOE, J-interactions).

Using the method of RDC measurement we established that the phenoxanthiin derivative[3] occurs in 1,2-alternate conformation (Fig. 1-A) while dinitroso-derivative adopts pinched cone conformation with nitroso groups on declined (flattened) aromatic rings (Fig. 1-B). In case of 2-pyridylsulfoxide calix[4]arene with intramolecularly bridged meta positions[4] we succeeded to specify the X-RAY structure (Fig. 1-C).

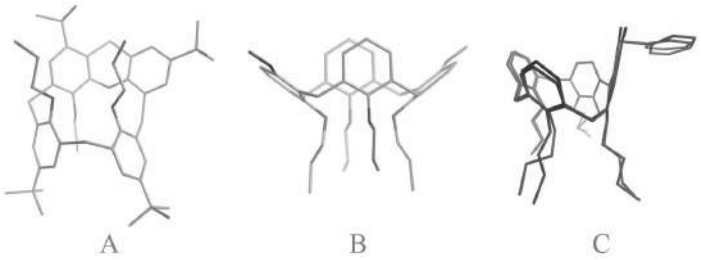

Figure 1: Structure of three types of new inherently chiral calix[4]arene derivatives obtained by the RDC measurement method. X-ray structure is shown of Fig. C in gray for comparison.

Acknowledgments: This research was supported by the Czech Science Foundation (P207/12/2027).

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An efficient approach to 6D HNCO(NCA)CONH

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Intrinsically disordered proteins due to their low chemical shifts dispersion are difficult target for NMR studies. The resonance assignment is usually performed using 4D and 5D experiments which are needed to resolve overlapping signals. Approaches using even more dimensions are believed to be very insensitive, and thus leading to very longmeasurement times. What is more, performance of experiments exploiting amide protons detection is additionally hampered by chemical exchange of well exposed to the solvent amide protons. In this study we present that optimized 6D HNCO(NCA)CONH experiment can be successfully acquired in about 13 hours using 800 MHz spectrometer with Room Temperature probe (15°C sample temperature was set to suppress amide protons exchange). Such experiment allow to obtain nearly all possible correlation signals using standard 1 mM α-Synuclein sample leading to a nearly full assignment of all non pre–proline residues.

Analysis of molecular mobility in pathogenic and protective mutants of human prion protein from ^{15}N relaxation data

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Prion diseases, also known as transmissible spongiform encephalopathies (TSE), are a group of neuropathies characterized by a fatal spongiform neurodegeneration of the brain, facilitated by conformational changes in the prion protein (PrP) observed in humans and other animals. However, the initiation and propagation mechanisms by which PrP convert from normal cellular form (PrP^{C}) to the protease resistant PrP^{Sc} form is still not understood in detail. One of the important factors of this phenomenon are processes of molecular dynamics which facilitate and control the conversion of PrP^{C} to PrP^{Sc} forms.

In the presented study, the backbone dynamics of the human pathogenic mutant (Q212P), protective mutant (Q218K), and two wild-type constructs PrP(90-231) and PrP(23-231) were analyzed at pH 5.5. The high resolution 3D structures of all mutants were solved previously in our group [1, 2] with the exception of the PrP(23-231) protein. There were several studies focused on analysis of ^{15}N relaxation data performed on mouse PrP protein [3, 4] demonstrated similarity of dynamic performed on the mouse wild-type and several PrP mutants. Our studies focused on human pathological and protective PrP mutants. Experimental data (^{15}N R_1, R_2 relaxation rates, and ^{1}H-^{15}N NOE) were acquired under two magnetic fields (16.4 T and 18.8 T) and analyzed with both Spectral Density Mapping and ModelFree formalisms. Moreover, results evaluated for the PrP(90-231) construct are analyzed together with whole length PrP(23-231) protein, which provide possibilities to explore tiny changes in dynamic processes caused by a long, unstructured N-terminal domain. Our studies reveal the possibility to explore the effects of molecular dynamics in the initial steps of the transformation of cellular PrP^{C} into the pathogenic PrP^{Sc} form.

Acknowledgments: This work was partially supported by Polish National Centre for Research and Development under research grant 178479 (contract number PBS1/A9/13/2012).

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